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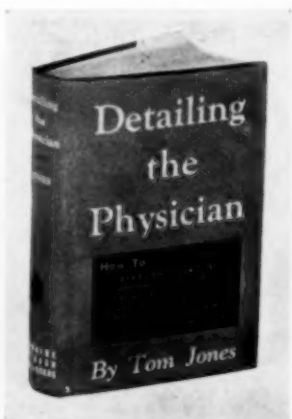

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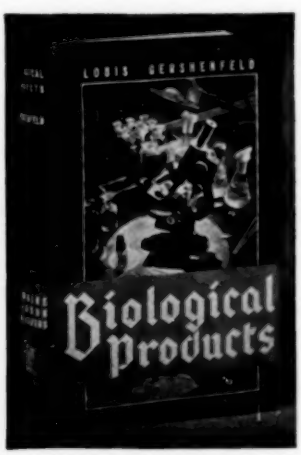

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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VOLUME XXX *MARCH, 1941* NUMBER 3 CONSECUTIVE No. 5

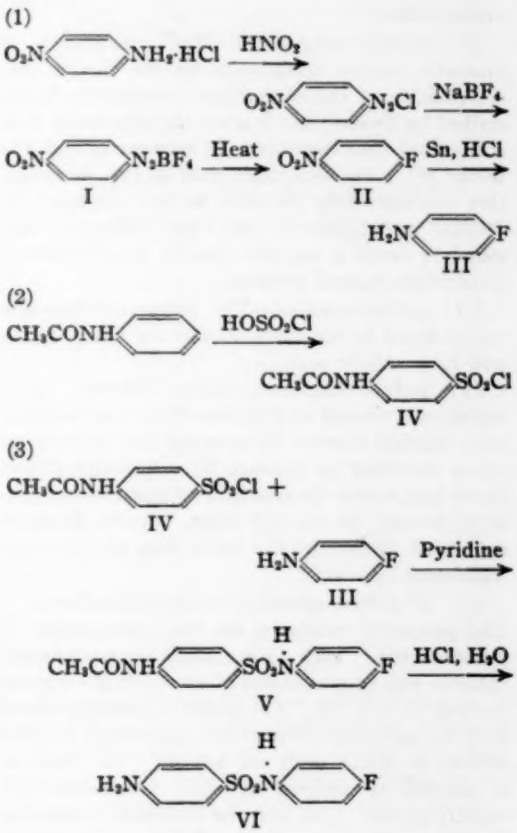
Synthesis and Toxicity of N^1-*p*-Fluorophenylsulfanilamide

By George P. Hager, E. B. Starkey and C. W. Chapman*

In reviewing the investigations of sulfanilamide derivatives, it was observed that the N^1-*p*-aminophenyl derivative has been reported to be twice as active and of the same order of toxicity as sulfanilamide (1). It was further noted at that time that there had been no reports of a fluorine derivative of sulfanilamide. In view of these facts the N^1-*p*-fluorophenylsulfanilamide was prepared as was the N^1-phenylsulfanilamide for purpose of comparison. As the investigation of these compounds was nearing completion, Suter and Weston (2) published a report on a series of fluorinated sulfanilamide derivatives among which was included the compound described in this paper.

The work here reported gives a procedure for the preparation of N^1-*p*-fluorophenylsulfanilamide in better yield and higher state of purity (as judged by melting points) than that obtained by the earlier procedure of Suter and Weston (2). There are also included the results of the experiments thus far completed to determine the relative toxicities of sulfanilamide, N^1-phenylsulfanilamide and N^1-*p*-fluorophenylsulfanilamide.

The N^1-*p*-fluorophenylsulfanilamide was synthesized according to the following scheme:



EXPERIMENTAL

I. p-Nitrobenzenediazonium Borofluoride.—The compound was prepared by Dunker, Starkey and

* Wm. R. Warner Fellow, 1939–1940, University of Maryland. Abstracted from a thesis submitted to the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Master of Science.

Jenkins (3) by an improved method taken from the work of Balz and Schiemann (4), involving the diazotization of *p*-nitroaniline in the presence of fluoboric acid. While better yields were obtained if the diazonium borofluoride was prepared directly using fluoboric acid, the method presented here obviates the necessity of preparing the fluoboric acid and utilizes the cheaper sodium fluoborate.

To 200 cc. of hydrochloric acid (sp. gr. 1.19) contained in a 2-l. beaker in an ice bath and stirred with a mechanical stirrer, were added 139 Gm. (1 mol.) of *p*-nitroaniline. The mixture was cooled to 5–10° C. While stirring vigorously, a solution of 69 Gm. (1 mol) of sodium nitrite in 150 cc. of water was added slowly from a separatory funnel, keeping the temperature of the mixture at 5–10° C. When the addition was complete, a cold filtered solution of 272 Gm. (2 mols) of sodium fluoborate in sufficient water was added to the solution of the diazonium chloride. The stirring was continued for fifteen minutes longer. The solid was filtered on a fritted glass funnel, washed once with alcohol and three times with ether and dried partially by drawing air through it. The resulting light yellow solid was dried over night in a desiccator over P_2O_5. The average yield was 92% of the theoretical based on the weight of *p*-nitroaniline taken.

II. p-Fluoronitrobenzene.—The preparation of aromatic fluorine compounds by the thermal decomposition of the diazonium borofluorides is described by Dunker and Starkey (5) who based their method on that developed by Schiemann and Pillarsky (6). The procedure used in this investigation was essentially the same as that employed by Dunker and Starkey. The chief difference consisted in carrying out this process at atmospheric rather than reduced pressure.

III. p-Fluoroaniline.—The *p*-fluoronitrobenzene was reduced in the usual manner by means of tin and hydrochloric acid.

IV. p-Acetaminobenzenesulfonyl Chloride.—Acetanilide was treated with chlorosulfonic acid and the acid chloride isolated by pouring the mixture on ice as described by Stewart (7). Although it was found best to use the crude damp material immediately because of decomposition, several attempts were made to prepare the stable pure product with indifferent success.

V. N^1-p-Fluorophenyl-N^4-Acetylsulfanilamide.—The procedure employed for the condensation of *p*-fluoroaniline with *p*-acetaminobenzenesulfonyl chloride was an adaptation of a more or less general method (1, 8, 9, 10). The amine is generally mixed with the acid chloride in molar proportions in some solvent in which both are soluble. The reaction is allowed to proceed at room temperature or slightly above. Care must be excerised in choosing the proper condensing agent for the hydrochloric acid liberated. When the amine is available and strongly basic, a mol in excess is used. When the amine is weakly basic, sodium bicarbonate or sodium acetate is used. When working with amino acids or nitro compounds, sodium hydroxide, potassium hydroxide or pyridine is necessary. Suter (2) prepared N^1-*p*-fluorophenyl-N^4-acetylsulfanilamide using water as a solvent and sodium carbonate as the condensing agent. Better yields were obtained when using acetone as solvent and pyridine as condensing agent as was done in the following procedure.

To a mixture of 5.6 Gm. (0.05 mol) of *p*-fluoroaniline, 12 Gm. (0.05 mol) of *p*-acetaminobenzenesulfonyl chloride and 40 cc. of acetone in a 1-l. round-bottomed flask with a reflux condenser, were added 8 cc. of pyridine. Heat was generated and a clear solution resulted. The mixture was allowed to stand with frequent shaking for three hours. At the end of this time it was poured slowly with stirring into two volumes of water. An oil settled out and solidified. The solid was filtered off and washed with water. The was either deacetylated immediately or purified by the following procedure. The crystals were dissolved by gentle heat in 2*N* sodium carbonate to separate them from any unchanged *p*-fluoroaniline. The alkaline solution was then acidified with 2*N* hydrochloric acid and the reprecipitated N^1-*p*-fluorophenyl-N^4-acetylsulfanilamide collected on a filter. Recrystallization from dilute ethanol produced an average yield of 80% of white crystals of N^1-*p*-fluorophenyl-N^4-acetyl sulfanilamide, melting at 190° C.

VI. N^1-p-Fluorophenylsulfanilamide.—The hydrolysis of the N^1-*p*-fluorophenyl-N^4-acetylsulfanilamide was carried out by essentially the same method as that used by Gelmo (11), modified by the addition of ethanol to dissolve the acetyl derivative. Suter (2) carried out this hydrolysis by refluxing with sodium hydroxide and obtained N^1-*p*-fluorophenylsulfanilamide in 47% yields and melting at 163–164° C.

A mixture of 15.5 Gm. (0.02 mol) of N^1-*p*-fluorophenyl-N^4-acetylsulfanilamide, 100 cc. of hydrochloric acid (sp. gr. 1.19), 225 cc. of ethanol and 50 cc. of water was placed in a 1-l. round-bottomed flask fitted with a reflux condenser. Heat was generated and a clear solution resulted. The mixture was refluxed for two hours, at the end of which time it was neutralized to litmus with sodium carbonate solution. The solid was collected on a Büchner funnel and washed free of carbonate with water. The solid was then dissolved in sufficient hot 2% aqueous sodium hydroxide, the solution diluted to 200 cc. with water and boiled with charcoal for two hours. The hot solution was carefully filtered and the treatment was repeated until the filtrate was only slightly yellow. Three volumes of water were added and sufficient 2% hydrochloric acid was slowly stirred into the mixture to make it neutral to litmus. The solid was filtered off, washed and recrystallized from 30% ethanol.

The N^1-*p*-fluorophenylsulfanilamide was obtained in flat white needles which sintered at 162–165° C., softened at 165° C., melted at 166.5° C.

(cor.). Sulfanilamide derivatives, in general, have been found to soften before they melt (1). The average yield is 55% of the theoretical based on the weight of *p*-fluoroaniline taken.

Analysis. Theor. for $C_{12}H_{11}O_2N_2SF$: 10.53% N. Found: 10.59% N.

TOXICITY

The procedure followed in measuring the relative toxicities of sulfanilamide, N^1-phenylsulfanilamide and N^1-*p*-fluorophenylsulfanilamide was based in part on the work done by Rosenthal (12). The drug was suspended in olive oil by trituration in a mortar. Dilutions were made such that approximately 0.5 cc. of an olive oil suspension would be injected into each mouse, depending on the body weight. It was, therefore, necessary to employ 1% to 20% suspensions. Both sexes of mice weighing from 18 Gm. to 22 Gm. were used. These were deprived of food about twelve hours prior to injection. The injections were made intraperitoneally and sealed in with collodion. After the injection, the animals were observed hourly for twelve hours. A record was made of the symptoms, the time of death and the autopsy findings.

Long (13) describes the symptoms of sulfanilamide poisoning as those of a bilateral vestibular dysfunction and spastic paralysis. The symptoms of poisoning with N^1-*p*-fluorophenylsulfanilamide resembled in character those of sulfanilamide. However, the transition from one phase of the syndrome to the next is accomplished in a shorter time in sulfanilamide poisoning and the symptoms are more pronounced. The first manifestation of poisoning is a weakness in the hind legs which is followed by ataxia. The movements on stimulation become convulsive and incoördinated. In many cases there is a tendency to symmetrical convulsions with the occasional appearance of opisthotonus. This continues through a stage of prostration during which the animal is more difficult to arouse and which in most cases is terminated by death.

The autopsy shows some significant differences between the action of sulfanilamide and N^1-*p*-fluorophenylsulfanilamide. The bladder of a sulfanilamide-poisoned animal is generally hard, small and filled with a thick white mass, while that of an animal poisoned with N^1-*p*-fluorophenylsulfanilamide is often distended with a clear yellow liquid. The heart in either case may be normal or slightly dilated. The lungs are more profusely hemorrhagic in the sulfanilamide-poisoned animal.

Table I.—Toxicity of Sulfanilamide by a Single Intraperitoneal Injection in Mice

Expt. No.	No. of Mice	Concn. of Suspn., %	Dose (Gm. per Kg.)	Percentage Mortality 12 Hr.	30 Hr.	46 Hr.	48 Hr.	54 Hr.
5	4	7	1.730	0	25	75	75	100
	4	9	2.280	0	50	75	100	100
	4	12	3.010	75	100	100	100	100
	4	16	3.960	100	100	100	100	100
9	4	5	1.225	0	0	0	0	0
	4	7	1.614	0	0	25	25	25
	4	9	2.133	0	0	25	25	75
	4	11	2.805	25	50	75	75	75
	4	15	3.698	100	100	100	100	100

Table II.—Toxicity of N^1-*p*-Fluorophenylsulfanilamide by a Single Intraperitoneal Injection in Mice

Expt. No.	No. of Mice	Concn. of Suspn., %	Dose (Gm. per Kg.)	Percentage Mortality 12 Hr.	30 Hr.	46 Hr.	48 Hr.	54 Hr.
5′	4	2	0.450	0	0	25	25	25
	4	2.5	0.593	0	0	25	25	50
	4	3	0.782	0	0	25	75	75
	4	4	1.030	0	0	50	100	100
	4	6	1.359	0	0	100	100	100
6	3	6	1.413	0	33	100		
	3	8	1.995	0	33	100		
	3	12	2.818	0	66	100		
	3	19	3.981	0	100	100		
8	4	5	1.189	0	25	75	75	100
	4	7	1.679	0	25	100	100	100
	4	10	2.371	0	50	100	100	100
	4	14	3.350	0	100	100	100	100
	4	19	4.732	0	100	100	100	100
10	8	2	0.540	0	0	0	0	0
	6	3	0.760	0	17	50	50	50
	6	5	1.070	0	17	100	100	100
	6	6	1.520	0	33	100	100	100
	8	8	2.140	0	75	100	100	100

The preliminary experiments indicated that the action of N^1-*p*-fluorophenylsulfanilamide is delayed, and that it would be technically impossible to determine the LD_{50} for a twelve-hour interval because of the concentration of the suspension it would be necessary to inject. It was also noted that the N^1-phenylsulfanilamide is still less toxic than the fluorinated compound in the twelve-hour interval. In order to compare these two substances, a 50% suspension of each in olive oil was prepared and an

amount of each corresponding to equal molecular quantities and representing the largest single dose that could be injected in 0.5 cc. volume was administered intraperitoneally to groups of eight mice. The doses employed amounted to 12.5 Gm. of N¹-*p*-fluorophenylsulfanilamide and 11.75 Gm. of N¹-phenylsulfanilamide per Kg. of body weight. Three out of eight mice receiving the fluorinated compound died in twelve hours and the remainder died in twenty-four hours. All the mice receiving the unfluorinated compound survived permanently with the exception of one which was found decapitated. From this experiment, it can be concluded that at the end of twelve hours both of these compounds are much less toxic than sulfanilamide and that the fluorinated compound is more toxic than the unfluorinated. The experiment further showed that under the experimental conditions employed, N¹-phenylsulfanilamide is so nontoxic that it would be impossible to determine an acute toxic dose given by single injection.

Experiments were then conducted to determine the LD_{50} of sulfanilamide and N¹-*p*-fluorophenylsulfanilamide at different time intervals. Some of the conditions of these experiments and the results obtained are given in Tables I and II. From the results the LD_{50}'s at different time intervals were calculated by Kärber's method (14) and are recorded in Table III. It will be noted that the

Table III.—Comparison of the LD_{50}'s, as calculated by Kärber's Method, for Sulfanilamide and N¹-*p*-Fluorophenylsulfanilamide

Expt. No.	Date	Time of Observation (Hours)	No. of Animals	LD_{50} (Gm. per Kg.) Sulfanilamide	LD_{50} (Gm. per Kg.) Fluorine Derivative
5	2/25/40	11	16	3.57	..
9	3/16/40	30	20	2.78	..
6	3/2/40	30	16	..	2.98
8	3/16/40	30	20	..	2.82
5′	2/25/40	46	20	..	1.07
9	3/16/40	48	20	2.90	..
10	4/21/40	48	34	..	0.98
9	3/16/40	54	20	2.48	..
5′	2/25/40	54	20	..	0.79

LD_{50} of sulfanilamide is practically constant for a thirty, forty-eight or fifty-four hours' observation period. While the LD_{50} of N¹-*p*-fluorophenylsulfanilamide is practically the same as that of sulfanilamide at the end of thirty hours, it drops to one-third at the end of fifty-four hours. The absolute values for the LD_{50}'s presented in the tables have large standard deviations and must be repeated employing more animals in order to determine accurately the significance of the observed differences. Several of the experiments in the tables were repeated in the summer when it was found that the relative LD_{50}'s remained the same but the absolute values changed slightly.

SUMMARY

1. N¹-*p*-fluorophenylsulfanilamide has been prepared and characterized. The product was obtained in purer state and better yield than that obtained by the method reported by Suter (2).

2. The acute toxicity of a single intraperitoneal injection in an olive oil suspension of the N¹-*p*-fluorophenylsulfanilamide has been compared to that of N¹-phenylsulfanilamide and sulfanilamide. The N¹-phenylsulfanilamide was found to be practically non-toxic by a single dose. The toxicity of sulfanilamide and N¹-*p*-fluorophenylsulfanilamide is essentially the same at the end of thirty hours, while at the end of fifty-four hours the fluorinated compound has a delayed toxicity of about three times that of sulfanilamide.

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Sir William Ramsay (1852–1916) was awarded the Nobel Prize for Chemistry in 1902 in recognition of his discovery of the individual gaseous basic materials in the atmosphere and the determination of their place in the periodic system.

Factors Affecting the Toxicity of Red Squill*

By J. A. Lubitz, A. S. Levine and C. R. Fellers†

INTRODUCTION

The object of this work was to determine some of the factors affecting red squill toxicity for the purpose of making red squill more effective as a rat poison. Experiments were conducted to determine the effect of the sex of rats, heat, moisture, fat, pectin and diet on the toxicity of red squill.

Winton (1), Munch, Silver and Horn (2), LeBlanc and Lee (3), and Crabtree, Ward and Welch (4) discuss the effect of red squill on male and female rats. Winton (1), O'Connor, Buck and Fellers (5), LeBlanc and Lee (3), and Munch, Silver and Horn (6) report on the effect of heat application to squill. O'Connor (7) found that white rats prefer dry rather than moist baits containing red squill powder. The effect of fats and oils on the action of drugs and poisons is discussed by Underhill (8) and Sollman (9). It is interesting to note the observation of Manville, Bradway and McMinis (10) that galacturonic acid from pectin can be utilized by rabbits in the detoxification of menthol. Winton (1) states that abnormal diets have little influence on the susceptibility of the rat to squill poisoning. Smith (11) and Gortner (12) report on the effect of diet on selenium toxicity.

EXPERIMENTAL

Procedure of Assay.—The standard assay technique for red squill powders (5, 6) was employed with the exception that all rats were starved for 24 hours instead of 18 hours. The lethal dose was taken to be the smallest amount of red squill powder in mg. per Kg. of body weight that killed 90 to 100 per cent of the rats within 120 hours.

SOME FACTORS INFLUENCING THE TOXICITY OF RED SQUILL

(*1*) *Sex Differences.*—The differences in toxicity of 2 red squill powders due to sex were determined by feeding at various levels to male and female rats on the basis of body weight.

When a powder called Reference Standard Red Squill of 1934 was fed to females only, the results showed that the toxic level of this powder was 350 mg./Kg. toward female albinos. In feeding this squill to male rats, it was found to be 800 mg./Kg. in toxicity.

The toxicity of a commercial red squill powder was calculated to be between 300 mg./Kg. and 400 mg./Kg. for female rats and between 700 mg./Kg. and 800 mg./Kg. for male rats.

The above experiments show that Reference Standard Squill of 1934 was 2.27 times more toxic for female than for male rats, and that the squill obtained commercially was about twice as toxic for females as for males. This work shows the fallacy of using female rats or rats of both sexes in an assay for red squill because of the greater resistance of male rats. For this reason a standard assay of red squill should be based on male rats.

(*2*) *Application of Heat.*—Heat is destructive to many organic toxicologic substances. To determine the effect of heat treatment on the toxicity of red squill the following experiment was conducted.

Reference Standard Squill of 1934, testing 800 mg./Kg. for male rats, was used in this work. The squill was spread in thin layers on aluminum trays and heated in an electric oven. The temperature to which the squill was heated was measured by means of a thermocouple which was placed in contact with the squill. One hundred Gm. portions of red squill were heated for 30 minutes at 100°, 125°, 150°, 175°, 200° and 225° C. Powdered dextrose was added to make up for any loss in weight due to evaporation of moisture or charring.

The toxicity determinations were performed using male rats which were starved for 24 hours, and then fed on an 800 mg./Kg. level with the heat-treated samples of squill in baits containing about two Gm. of Fox Chow as carrier.

This experiment indicated that the higher the temperature applied to the squill powder, the less toxic it becomes. When heated at 100° C. for one-half hour in a hot air oven, the squill was only slightly changed in toxicity, while at 225° C. it was rendered non-toxic to male rats at the 800 mg./Kg. feeding level. These results are expressed in Table I.

Table I.—Effect of a 30-Minute Heating Period on the Toxicity of Standard Red Squill Fed at an 800 Mg./Kg. Level to Male Albino Rats

Temperature of Heat Treatment of Squill, ° C.	Result, Rats Killed/Rats Fed
100	7/10 (2 rats very ill)
125	6/10 (1 rat ill)
150	1/10
175	3/10
200	3/9
225	0/9

* Contribution No. 369 Massachusetts Agricultural Experiment Station, Amherst.

† Adapted from a thesis presented to the Graduate School of the Massachusetts State College by J. A. Lubitz for the degree of Master of Science.

(*3*) *Moisture in Squill Baits.*—In order to determine the effect of moisture on the toxicity of red squill baits, the following experiment was conducted.

Baits containing a lethal dose of Reference Standard Squill (5 per cent moisture) and Purina Fox Chow (8.46 per cent moisture) as a base were mixed with 0, 25, 50, 75 and 100 per cent of the rats' daily requirement of water. The average daily requirement was found to be about 190 cc. per Kg. for females and about 140 cc. per Kg. for male rats. These baits were fed to rats that had been starved and deprived of water for 12 hours. Female rats were fed on a 350 mg./Kg. level and male rats on an 800 mg./Kg. level of this squill.

It can be seen from the results shown in Table II that the amount of moisture in a bait bears no relationship to the toxicity of the bait.

Table II.—Influence of Water Content of Baits on Toxicity of Standard Red Squill

Amount of Water in Bait Per Cent of Daily Requirement	Rat Sex	Squill Level Mg./Kg.	No. of Rats Leaving Some Bait	Result, Rats Killed/ Rats Fed
0	M	800	1	4/5
0	F	350	0	5/5
25	M	800	0	5/5
25	F	350	0	5/5
50	M	800	1	5/5
50	F	350	3	4/5
75	M	800	2	4/5
75	F	350	5	2/5
100	M	800	5	3/5
100	F	350	5	1/5

(*4*) *Fat.*—Fats and oils have a retarding effect on the rate of absorption of certain poisons; they also decrease the toxic effect of these poisons on animals due to some purely physical action.

Hydrogenated fat (Crisco) and petrolatum were used in experiments to determine the effect of fat on the toxicity of red squill to rats. Each rat was fed a bait consisting of a known amount of powdered squill, a known percentage of fat and Fox Chow when necessary to make 1 per cent of the rat's body weight. These were fed the required percentage of fat and enough Fox Chow to equal 1 per cent of their body weights. The control rats evidenced no harmful effects from these baits which contained no squill.

It is seen from the results shown in Table III that fat lowers the toxicity of red squill. It is interesting to note that an increase in the dose of red squill from 350 mg./Kg. to 400 mg./Kg. overcame any protective action that the Crisco baits had on female rats.

(*5*) *Pectin.*—It is thought that pectin acts as a detoxifying agent because of its physical character and because on being broken down in the organism it gives rise to galacturonic acid. This acid is capable of forming conjugation products with toxic materials in the same manner as glucuronic acid (10). To determine the effect of pectin on red squill the following experiment was conducted.

Baits were prepared containing 10 and 50 per cent of dry citrus pectin. They were so prepared that when fed in the proportion of 1 per cent of a rat's body weight, they would contain an 800 mg./Kg. dose of Reference Standard Squill along with the desired percentage of pectin with Fox Chow as a carrier.

When male rats were fed an 800 mg./Kg. dose of the squill in a bait containing 10 per cent pectin, 7/10 died. When 50 per cent pectin was fed in a like bait, 3/8 died. Ten rats were used on the 50 per cent pectin bait, but as 2 rats refused to take the bait, they were not considered in the experiment (Table IV).

Table IV.—Effect of Pectin on the Toxicity of Reference Standard Red Squill Fed on an 800 Mg./Kg. Level to Male Rats

Pectin in Bait %	Result, Rats Killed/Rats Fed
10	7/10
50	3/8

This preliminary experiment indicates that pectin has a detoxifying action on red squill. It seems that the more pectin in a red squill bait, the less toxic will the bait be to rats.

Table III.—Effect of Fats on the Toxic Action of Reference Standard Red Squill of 1934 toward Albino Rats

Type of Fat	Added Fat in Bait, %	Red Squill Feeding Level Mg./Kg.	Rat Sex	Result, Rats Killed/Rats Fed
Control (no Crisco)	0[a]	350	F	10/10
Crisco	10	350	F	7/10 (2 rats ill)
Crisco	25	350	F	8/10 (2 rats ill)
Control (no Crisco)	0	400	F	10/10
Crisco	10	400	F	3/3
Crisco	75	400	F	4/4
Crisco	96	400	F	3/4
Control (no Crisco)	0	800	M	9/10
Crisco	10	800	M	7/10
Control (no petrolatum)	0	350	F	10/10
Petrolatum	10	350	F	7/10 (3 rats very ill)
Petrolatum	25	350	F	6/10 (2 rats ill)

[a] No added fat, fat content of ration 3.0%.

In addition to the control rats fed on the different levels of squill in baits without fat, control rats were used on all percentages of Crisco and petrolatum.

(*6*) *Diet.*—The action of poisons is sometimes affected by diet. The question was raised whether or not dietary factors might in some way affect the

toxicity of red squill. Preliminary experiments showed that considerable fat in red squill baits slightly lessens the toxicity of the baits. In feeding baits containing 92 per cent protein (casein) and 8 per cent of Reference Standard Red Squill of 1934 (800 mg./Kg. dose) to male rats in amounts equal to 1 per cent of their body weights, there was no change in red squill toxicity. Carbohydrate (cornstarch) was fed in like manner with identical results.

In an attempt to answer the question as to whether the type of diet has any effect on the killing power of red squill, diets were prepared as follows:

Diet I.—Low protein, high carbohydrate diet, consisting of 7 per cent protein, 73 per cent carbohydrate, 11 per cent fat.

Diet II.—High protein, low carbohydrate diet consisting of 45 per cent protein, 35 per cent carbohydrate, 11 per cent fat.

Diet III.—Low protein, high fat diet, consisting of 7 per cent protein, 37 per cent fat, 47 per cent carbohydrate.

Diet IV.—The control diet, Fox Chow, consisting of 20 per cent protein, 3 per cent fat, 46 per cent carbohydrate.

The composition of these experimental diets is shown in Table V. The rats used were normal male albino rats which had been maintained on a diet of Purina Fox Chow. Rat weights ranged from 100 to 320 Gm. Four groups of rats were fed the various diets over a period of 1 month. They then were left without food for 24 hours, after which they were fed an 800 mg./Kg. dose of Reference Standard Red Squill of 1934 in enough Fox Chow to equal 1 per cent of the rat's body weight. Fox Chow was used as the carrier rather than the experimental diets in order to minimize the physical interference which the diets might have on the toxicity of the squill.

Table V.—Percentage Composition of Experimenta Diets

	Diet Number			
Component	I, %	II, %	III, %	IV,[a]
Commercial casein	7	45	7	Purina Fox Chow
Cornstarch	73	35	47	
Crisco	9	9	35	
Cod liver oil	2	2	2	
Dried brewers yeast	5	5	5	
Osborne and Mendel salt mixture	4	4	4	

[a] Guaranteed analysis of Purina Fox Chow; crude protein 20%, nitrogen free extract 46%, crude fat 3% and crude fiber 6%. It also contains vitamins and minerals.

The results shown in Table VI are described in the following paragraphs.

Table VI.—Effect of Previous Diet on the Toxicity of Standard Red Squill Fed in an 800 Mg./Kg. Dose to Male Rats

Type of Diet Fed for 30 Days Previous to Squill Feeding	Result, Rats Killed/Rats Fed Squill
Low protein, high carbohydrate	8/9
High protein, low carbohydrate	2/10
Low protein, high fat	7/10
Control (Purina Fox Chow)	8/9

Diet I.—Ten rats were used in this experiment. One rat died at the end of 10 days on this diet, cause unknown, and, therefore, was not considered in the results. These rats appeared normal after 30 days on this diet. When fed squill 8/9 died, indicating that this diet has no influence on the toxicity of red squill.

Diet II.—Ten rats were used. These rats appeared normal after 30 days of this diet. They were then fed squill. Two out of 10 rats died, indicating that a high protein, low carbohydrate diet markedly decreases the toxicity of red squill.

Diet III.—In this group there were 10 rats. After 30 days these rats developed a greased appearance, but otherwise appeared normal. When fed the squill 7/10 rats died, indicating that this diet has a very slight effect on the toxicity of red squill. This slight effect is due perhaps to the amount of fat remaining in the alimentary tract of the rats because of the previous high fat diet. This resulted in spite of the fact that they were without food for 24 hours. In a previous experiment it was shown that large amounts of fat have a slight lessening effect on the toxicity of red squill.

Diet IV.—Ten rats were placed on this control diet. One rat died from causes unknown at the end of 29 days, and was not considered in this experiment. All nine rats appeared normal at the end of 30 days. On being fed the squill 8/9 died, indicating that this diet had no effect on the toxicity of red squill.

Experiments on rats indicate that the toxicity of red squill is affected by dietary factors. It is to be noted first that one-bait feedings of 92 per cent protein and 92 per cent carbohydrate with a lethal dose of squill have no effect on the toxicity of the squill; while one-bait feedings of various percentages of fat fed in like manner cause a slight decrease in the toxicity of squill. High carbohydrate, low protein; and high fat, low protein diets when fed to rats for 30 days seem to have no marked effect on the toxicity of red squill fed on the 31st day. However, a high protein (casein), low carbohydrate diet when fed in like manner has a marked effect in decreasing the toxicity of red squill.

The reason for the important role of protein (casein) in the diet on the toxicity of red squill is unknown. It may be due to raised tissue immunity toward red squill caused by the high protein diet, or to the combination of the poison with degradation products of protein present in the rat due to the diet.

SUMMARY AND CONCLUSIONS

1. Red squill is about half as toxic for males as it is for female rats. It is recommended that standard assays of red squill be based on male rats rather than female rats, or rats of mixed sexes.

2. The higher the dry air temperature

(above 100° C.) applied to red squill powder, the less toxic it becomes.

3. The amount of moisture in a red squill bait has little relationship to the toxicity of the bait.

4. Large percentages of protein (casein) or carbohydrate (cornstarch) in squill baits do not affect the toxicity of the baits. Considerable fat in red squill baits results in a slight decrease in the toxicity of the bait. The more pectin in a red squill bait, the less toxic will the bait be to rats.

5. Low protein diets containing (1) high fat and (2) high carbohydrate when fed to rats for 30 days, seem to have no marked effect on the toxicity of red squill. However, a high protein (casein), low carbohydrate (cornstarch) diet has a rather definite effect in decreasing the toxicity of red squill baits.

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Svante August Arrhenius (1859–1927) was awarded the Nobel Prize for chemistry in 1903 in view of the special value of his theory of electrolytic dissociation in the interest of the development of chemistry.

Pyrethrin Content of Pyrethrum Grown in India

*By J. K. Lahiri, S. Ghosh and R. N. Chopra**

Pyrethrum flowers imported into India are from *Chrysanthemum cinerariæfolium*, which is the only species that is commercially important. The product imported is either the powdered flowers or their extracts or various preparations made from either of these. They are widely used as household insecticides, livestock sprays and horticultural dusts and sprays. Large quantities of pyrethrum or its preparations are now being used in India, where the control of disease due to insects is an acute problem, and in view of the vast economic possibilities for pyrethrum, attempts are being made to cultivate the plant in India. The experimental cultivation of pyrethrum flowers has been tried in Kashmir, Muree Hills, Kulu Valley, North Western Frontier Province, Kurrum Valley, United Provinces and other places, and it has been found that the plant grows well at altitudes of 5000–6000 ft. Very recently, however, pyrethrum has been grown successfully by Mr. S. N. Bal, Curator, Calcutta Museum, in the Mayurbhanj State (Orissa) at altitudes of 3400–3600 ft., and we are grateful to him for sending us two of these samples for our analyses. We are also thankful to Mr. R. L. Badhwar for securing samples of pyrethrum, grown in Kashmir and Murree Hills, from time to time for the present work.

The chemical assay of pyrethrum flowers depends upon the estimation of the insecticidal principles, pyrethrin I and pyrethrin II, which are the esters of the ketone-alcohol pyrethrolone with the two acids chrysanthemum monocarboxylic acid and chrysanthemum dicarboxylic acid methyl ester, respectively. The content of pyrethrin of the Dalmatian flowers ranges from 0.38% to 0.58%, the Japanese flowers from 0.58% to 1.21%, the Kenya flowers from 0.90% to 1.48%, the Californian flowers about 1.10%, the Spanish flowers about 0.57%,

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Table I.—Pyrethrin Content of Pyrethrum Flowers

Species	Source	Crop Year	Grade	Pyrethrins Per Cent
C. cinerariæfolium	Kashmir	1938	Open	0.702
C. cinerariæfolium	Kashmir	1939	Open	0.741
C. cinerariæfolium	Murree	1939	Open	1.300
C. cinercriæfolium	Murree	1939	Half-open	1.030
C. roseum	Murree	1939	Open	0.247
C. cinerariæfolium (Kenya seeds)	Mayurbhanj	1940	Open	1.154
C. cinerariæfolium (Harpenden seeds)	Mayurbhanj	1940	Open	1.138
C. cinerariæfolium	Kashmir (Tangmarg)	1940	Open	0.962
C. cinerariæfolium	Kashmir (Baramulla)	1940	Open	0.904
C. cinerariæfolium	Palampur	1940	Open	0.904
C. cinerariæfolium	Palampur	1940	Half-open	0.834
C. cinerariæfolium	Palampur	1940	Closed	0.491
C. cinerariæfolium	Kulu	1940	Open	0.702
C. cinerariæfolium	Kulu	1940	Half-open	0.734

and the Russian flowers about 0.24% (1). The method followed in these assays was that of Gnadinger and Corl (2) and the same method has also been used by us with very slight modifications to suit our laboratory conditions.

EXPERIMENTAL

Ten to thirty-five grams of pyrethrum flowers, powdered and passed through a 40-mesh sieve, were extracted for five hours with petroleum ether (B. P. 40–60° C.) in a Soxhlet extraction apparatus. The petroleum ether extract, which was less than 100 cc., was allowed to stand over night at room temperature. The solvent was then driven off at a temperature not exceeding 75° C. and the residue was immediately transferred with five portions of boiling aldehyde-free alcohol to a 100-cc. volumetric flask, using sufficient boiling alcohol to make the volume 80–85 cc. To the hot solution, 15 cc. of basic lead acetate were added and the flask shaken vigorously for a few minutes. After allowing it to cool, the solution was made up to the mark with alcohol. It was filtered through a Büchner funnel and anhydrous sodium carbonate (2 Gm.) was added to the filtrate. The flask was shaken frequently and was allowed to stand for ten minutes. After filtration, 10 cc. of the clear filtrate were taken in a Folin tube and 6 cc. of alkaline copper solution added. In another Folin tube 10 cc. of a standard dextrose solution (2 mg. of dextrose) were taken and 6 cc. of the same alkaline copper solution added. The tubes were placed upright for 45 minutes in a constant temperature water bath, set at 78° C. and controlled within 0.5°. After removal of the tubes from the water bath and cooling, 10 cc. of Folin reagent were added to each. After shaking, the contents were transferred separately to 100-cc. volumetric flasks and the solutions were made up to the mark with water. The solution from the pyrethrum extract was filtered through a Gooch crucible fitted with a heavy asbestos pad. The solutions were compared in a Klett colorimeter and from the readings the percentage of the pyrethrins was calculated in the usual way.

CONCLUSIONS

The samples of the commercial variety (*C. cinerariæfolium*) grown in India show a high content of the active constituents compared to imported and foreign samples.

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The Effect of Alkalinity or Acidity on the Stability of Ether*

By A. W. Berry and E. S. Herlong†

The United States Pharmacopœia requires that anesthetic ether be free from aldehyde and peroxide when tested by tests of high sensitivity, thus justly limiting the possible presence of these substances to extremely minute amounts. Consequently ether cannot develop more than minute traces of these impurities upon aging, if the ether is to remain in compliance with pharmacopœial specifications.

A copper-lined can has been shown to be effective in preventing deterioration of ether (1). Notwithstanding this fact, it seemed worth while to determine whether or not very mild alkalinity or acidity would have any bearing on the stability of ether and perhaps provide still other means of

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† Presented to the Scientific Section of the A. PH. A., Richmond meeting, 1940.

avoiding the development of aldehydes and peroxides on aging.

EXPERIMENTAL

Preliminary work included the preparation of ether made slightly alkaline with ammonia, and of ether mildly acidulated with glacial acetic acid and also sulfuric acid; and the packaging of these ethers in glass bottles, copper-lined cans and tin-lined cans. These samples were examined after three months, eleven months and forty-one months. The acidified ether in all three types of containers showed the presence of aldehydes each time the ethers were tested, while the ammoniated ether did not. These experiments indicated that a condition of mild acidity is unfavorable and one of mild alkalinity favorable for the protection of ether.

In extending this work ethers containing 0.00176, 0.00088, 0.00044, 0.00022 and 0.00011 Gm. of ammonium hydroxide per 100 cc. of ether were prepared and packaged in copper-lined and tin-lined containers. These were stored at room temperature and also at elevated temperature for thirty-three months. Tests showed that 0.00022 Gm. of ammonium hydroxide per 100 cc. was the optimum amount and that, with the other concentrations, the results were not as good.

Subsequently, an ether containing this optimum concentration of ammonia was packaged in copper-lined containers and tin-lined containers. At the same time a normal anesthetic ether was packaged in the same types of containers. These ethers were stored both at room temperature and at elevated temperatures for thirty-three months. They were subjected to the U. S. P. XI tests and also to other still more sensitive tests for aldehydes and peroxides (2). No practical difference was found between the ammoniated ether and the normal ether when packaged in copper-lined containers, but in the tin-lined containers the ammoniated ether showed less development of aldehydes.

At the same time that we studied ethers made alkaline with ammonia, we also studied the effect of other alkalinizing agents, such as monoethanolamine, triethanolamine, alcoholic potassium hydroxide and alcoholic sodium hydroxide. These likewise were packaged in both types of containers and stored for thirty-three months at both room temperature and elevated temperatures. Of this group, monoethanolamine was found to be superior to the other members in the preservation of ether, but was not as good as ammonia.

SUMMARY

From the work carried out, it has been found that faintly alkaline ethers when stored in copper-free containers are superior in stability to regular U. S. P. XI ether and to those which have been faintly acidified. The ethers faintly alkalinized with ammonia are superior in keeping qualities to ether made faintly alkaline with other agents. Faintly ammoniated ethers are slightly more stable in copper-lined containers under adverse storage condition than in the other types of containers studied. The protective effect of tinned iron containers is more readily demonstrated than that of copper-lined containers, because of the difficulty of causing any appreciable aldehyde development, even under severely adverse storage conditions, when ether is stored in copper.

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Color Reactions of Some Aliphatic Acids

*By George Roeder**

In the course of some other research work, I made the unexpected observation that alkali citrates, heated in acetic anhydride, give deep red colored solutions. In using acetic acid instead of the anhydride the solutions remained colorless.

The first phase of this reaction probably is the formation of alkali acetate and a mixed anhydride of acetylated citric and acetic acid. Since citric acid dissolved in hot acetic anhydride gives a colorless solution, it is clear that the alkali acetate formed in the first phase of the above reaction brings about the condensation of the mentioned mixed anhydride into the colored compound. Therefore, a solution of citric acid in hot acetic anhydride, with the addition of alkali acetate, should give the coloration. This

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is actually what happens. Furthermore, the same color reaction is observed when alkali salts of any organic acid are added instead of alkali acetate, because in every case alkali acetate is formed from the acetic anhydride and the salt of the organic acid. Citric acid dissolved in hot acetic anhydride gives a deep red solution on adding a few grains of alkali acetate, alkali formate, alkali benzoate, alkali succinate, etc., also when boiled with powdered flint glass.

In principle, the above reaction has been known for several years, since Fuerth and Herrmann (1) and later Casares-Lopez (2) described color reactions of citric, tartaric, aconitic, tricarballylic and agaricic acids with a mixture of 35 parts of pyridine and 15 parts of acetic anhydride. Under these conditions tartaric acid gave an emerald-green, citric acid a carmine, aconitic acid a purplish red and agaricic acid a purplish brown coloration.

Color reactions of aconitic acid and of malonic acid with acetic anhydride alone, without addition of basic compounds, have been described before by Taylor (3) and by Kleemann (4).

According to Fuerth and Herrmann, when pyridine is replaced by quinoline or other organic bases, only yellow or brown colors are obtained. This is an error probably caused by the large excess of bases employed by the authors. In fact, the color reactions of all the acids mentioned take place with practically every tertiary base, with trialkylamines and diethylaminoethanol, with quinoline, nicotine, cinchona alkaloids, strychnine, dimethylaniline, etc., when some drops or grains of these compounds are added to the solutions of the acids in acetic anhydride.

Now, the fact that the color reactions occur with alkali acetates and with trialkylamines excludes the assumption of the previous authors that the colors are produced by condensation of the acids with pyridine. Apparently, the basic compounds serve only as a means of condensation and perhaps to form salts or some other kind of addition products.

Among the various transformation products of citric acid, acetonedicarbonic acid has not been tested by the previous authors. Filling this gap, I have found that this acid, under the described conditions, also gives a splendid color.

In addition, I have succeeded in extending these reactions to a new class of compounds, *e. g.*, to lactones of sugar acids. Ascorbic acid and *d*-iso-ascorbic acid, glucono-*d*-lactone and glucoheptonic lactone have also been found to give coloration.

The chemical constitution of the colors is unknown. They are said to be of very high molecular weight (3000) (1). Their formation from common natural products by a simple condensation process seems to be of biological interest.

EXPERIMENTAL

The shade of the colors depends upon temperature, heating time, quantity of added base and upon the presence of other solvents mixed with the acetic anhydride. Therefore it is understandable that previous statements are partly incomplete and sometimes contradictory. For this reason, in addition to the description of the new color reactions, I give in the following a more complete report of the reactions previously known.

The tests were made each with 50 mg. of acid in 10 cc. of acetic anhydride (95%) adding a few grains of solids or about three drops of liquid bases.

Malonic Acid.—The solution in acetic anhydride when carefully heated first becomes yellow, then orange colored with green fluorescence, as described by Kleemann (4). On adding sodium acetate, pyridine, triethylamine or dimethylaniline and with further heating the color changes into a beautiful ruby-red with increased fluorescence.

Under the above conditions ethylmalonic acid does not give any color reaction.

Aconitic Acid.—The hot solution in acetic anhydride shows first an amethyst and later a green coloration (Taylor) (3). The addition of potassium acetate, pyridine, dimethylaniline, strychnine or triethylamine transforms the color into a deep purple.

Citric Acid.—A deep red color results when a few grains or drops of the following compounds are added to the hot solution of the acid in acetic anhydride: alkali acetates or alkali salts of other carboxylic acids, trialkylamines, pyridine, quinoline, strychnine, nicotine, cinchonine, diethylaminoethanol, triethanolamine, etc. When the acid is dissolved in a mixture of equal parts of acetic acid and acetic anhydride, a beautiful transparent red solution with a bluish shine is obtained after the bases have been added.

Acetyl citric anhydride reacts in the same manner. Alkali citrates give the coloration when heated with acetic anhydride alone.

Agaricin (Cetyl Citric Acid).—Commercial samples of different origin were of different purity. Some left an insoluble residue when dissolved in ethyl acetate, some gave colorations without addition of bases. The best available compound of foreign origin (Eimer & Amend) gave the following colors in boiling acetic anhydride: with alkali acetates and with triethylamine ponceau red, green fluorescence; with pyridine, light amethyst color; with nicotine or strychnine, deep purple; with dimethylaniline, yellow or yellowish green. The intensity of the colors produced by the alkali acetates increased progressively from lithium to caesium.

Tartaric Acid.—Alkali acetates, triethylamine, quinoline, nicotine, strychnine, dimethylaniline, pyridine boiled with the solution of the acid in acetic anhydride give successively a yellow, orange and finally a brown-red color. Increasing the quantity of pyridine changes the color into a deep green.[1]

Incidentally, this green color has been previously observed as an inconvenient side result in the preparation of hydroxy-maleinanhydride from diacetyl tartaric anhydride with pyridine (5).

Acetonedicarbonic Acid.—The freshly prepared acid in acetic anhydride gives successively yellow, orange, red and finally brown solutions when alkali acetates or organic bases are added.

Ascorbic Acid.—When the solution in hot acetic anhydride is boiled with a few grains of sodium acetate or potassium acetate, with triethylamine or nicotine, a deep red color with a bluish shine and slightly fluorescent results. When boiled with some drops of pyridine, the solution remains colorless. Dissolving the vitamin in one or two cc. of pyridine, adding acetic anhydride and boiling gives an orange color which deepens with prolonged heating into reddish brown.

d-Iso-ascorbic Acid acts like vitamin C.

Glucono-d-lactone.—The addition of some grains of potassium acetate or caesium carbonate, nicotine or triethylamine to the boiling solution in acetic anhydride produces a bright brown color. When the quantity of the base is increased and heating continued, a deep brown color is obtained. The reaction is much less intense with sodium acetate and nearly zero with the lithium salt.

Glucoheptonic Lactone.—Because of its insolubility in acetic anhydride the lactone is first covered with some pyridine and after warming slightly acetic anhydride is added. This solution remains colorless upon boiling. When caesium carbonate is added and boiling continued, a wine-red color first appears which gradually darkens into reddish brown and dark brown.

[1] The described color reactions of citric and tartaric acid are very suitable for the detection of these acids in aspirin preparations to which they are sometimes added (especially in hot countries) to disguise the ferric chloride reaction of a more or less decomposed aspirin.

Hydroxy-dimethylbutyrolactone.—This constituent of pantothenic acid did not give any visible reaction when boiled with acetic anhydride and basic compounds.

SUMMARY

1. The color reactions of some aliphatic polycarboxylic acids with acetic anhydride and basic compounds, described in the literature, are produced by a modified method.

2. The new method permits the correction of several errors made by earlier authors and complements the picture of these color reactions.

3. New color reactions are described for acetonedicarbonic acid and for a new type of compounds, of lactones of sugar acids, *e. g.*, gluconolactone, vitamin C, iso-ascorbic acid and glucoheptonic lactone.

My thanks are due Dr. E. Fernholz, former Director of the Chemical Laboratory of the Squibb Institute for Medical Research for a sample of hydroxy-dimethylbutyrolactone and to Chas. Pfizer & Co. who generously provided me with vitamin C, iso-ascorbic acid, gluconolactone, aconitic and tricarballylic acid.

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"The doorstep to the temple of wisdom is a knowledge of our ignorance"—Spurgeon

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Jacob Hendrick van't Hoff (1852–1911), the founder of stereo-chemistry, was awarded the first Nobel Prize for Chemistry in 1901 for his discovery of the laws of chemical dynamics and osmotic pressure in solutions.

A Preliminary Study of Artemisia Filifolia Torrey*

By Howard Hopkins† and Joseph B. Burt‡

Few of the plants native to Nebraska have been given any attention with regard to chemical investigation. Inasmuch as one of the most widely distributed plants in western Nebraska is *Artemisia filifolia* Torr., this plant suggested itself as one worthy of investigation. A specimen of the plant under consideration was identified by Professor T. J. Fitzpatrick, Curator of the Herbarium of the University of Nebraska, as *Artemisia filifolia* Torr. So far as could be learned, no previous chemical investigation of this plant has been reported.

EXPERIMENTAL

A. Collection of Material

On October 15, 1938, 130.07 Kg. of *Artemisia filifolia* Torr. were collected in Keith County, Nebraska. Only the green, tip ends of the plant were gathered. This was accomplished by gathering up a handful of the ends and breaking them off with the aid of a large knife or by clipping them off with a pair of shears. The material was packed in paper bags and shipped to Lincoln *via* truck, arriving five days after the material had been gathered. Of the 130.07 Kg. of fresh plant collected, 112.37 Kg. were allotted for steam distillation and 17.70 Kg. were reserved for drying.

During the first week of August of 1939, 186.10 Kg. of flowering *Artemisia filifolia* Torr. were collected in the same vicinity as was the 1938 crop; the 1939 crop was handled and shipped in the same fashion as was the 1938 crop. All the material collected in 1939 was subjected to steam distillation.

B. The Shrub

As previously indicated, 17.70 Kg. of the fresh plant were set aside for purposes of investigating the plant itself. This material had lost 20.40 per cent of its green weight by the time it was delivered in Lincoln. The material was spread out on the floor in a thin layer and allowed to dry for five weeks in the open air. At the end of this drying period, the leaves and flowering heads were stripped from the stems and separated therefrom. The leaves and flowers were ground in a Wiley mill, using the fine screen. The stems were similarly ground.

Table I.—Loss after Drying and Grinding

	Weight in Kilos			
	Fresh Plant	Upon Receipt	After Drying	After Grinding
Stems	...	...	3.25	3.18
Leaves	...	...	7.96	7.46
Stems and leaves	17.70	14.10	11.21	10.64

	Loss in Weight	
Stems and Leaves	In Kilos	In Per Cent
Upon receipt	3.60	20.40
After drying	6.49	36.70
After grinding	7.06	40.00

The ground stems were light tan in color and had a woody odor; the ground flower-leaf portion was gray in color and had an eucalyptol-like odor.

1. Moisture Determinations

The moisture content of the ground leaves and flowers as well as the ground stems was determined according to the toluene method of "The Pharmacopœia of the United States of America" (1). The water obtained from the moisture determinations was acidic to litmus paper. The percentages of water in the samples are shown in Table II.

Table II.—Percentages of Water in Air-Dried Plant

	I	II
Leaves and flowers	7.36	7.34
Stems	7.36	7.36

2. Material Volatile at 105° C.

Ten-gram samples of the flower-leaf portion and of the stem portion were placed in an oven heated to 105° C. Weighings were made at twenty-four hour intervals until a constant weight was reached. After about eight days of drying, the samples had lost all perceptible odor. Constant weight was reached at the end of three weeks of drying. The percentages of material volatile at 105° C. are shown in Table III.

Table III.—Percentages of Material Volatile at 105° C.

	I	II
Leaves and flowers	11.68	11.80
Stems	8.61	8.39

3. Ash Determinations

The ash content of the leaf-flower portion and the stem portion was determined according to methods of "The Pharmacopœia of the United States of America" (1), supplemented by methods outlined by Woodman (2). The results of these determinations are listed in Table IV.

Table IV.—Percentages of Ash in Air-Dried Plant

	Total Ash	Water-soluble Ash	Water-insoluble Ash	Acid-soluble Ash	Acid-insoluble Ash
Leaves and flowers					
I	6.35	1.27	5.08	3.26	1.82
II	6.23	1.35	4.88	3.18	1.70
III	6.42	1.41	5.01	3.11	1.90

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* An abstract of a thesis submitted by Howard Hopkins in partial fulfilment of the requirements for the degree of Master of Science, June 1940.

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‡ Professor of Pharmacy, University of Nebraska.

Table II. (*Continued*)

	Total Ash	Water-soluble Ash	Water-insoluble Ash	Acid-soluble Ash	Acid-insoluble Ash
Stems					
I	3.41	1.14	2.27	1.91	0.36
II	3.34	1.00	2.34	1.75	0.59
III	3.35	1.01	2.34	1.78	0.56

4. EXTRACTION WITH SELECTIVE SOLVENTS

Only the flower-leaf portion of the plant was subjected to extraction with selective solvents. Three four-hundred-gram samples of the flower-leaf portion were extracted with a series of selective solvents according to a modification of the method as originally proposed by Dragendorff (3).

The solvents employed, in the order in which they were applied, were as follows: petroleum ether, ether (tech.), chloroform (tech.), alcohol, water, one per cent aqueous hydrochloric acid solution and two-tenths per cent aqueous sodium hydroxide solution. Extraction with the first four solvents (petroleum ether, ether, chloroform and alcohol) was carried out in an extraction apparatus similar to that proposed by Wester (4) for continuous extraction; the temperature of the solvent in contact with the sample was somewhat below the boiling point of the solvent during these extractions. The water and hydrochloric acid extractions were carried out in open beakers at the temperature of the boiling solvent. The sodium hydroxide extraction was made at a temperature of about 75° C. Constant stirring was employed during this extraction in order to prevent superheating of the bottom of the beaker; breakage of a number of beakers resulted from this cause prior to the employment of mechanical stirrers.

The various extractions were carried to practical completion as was indicated by the absence of color in the solvent or the absence of residue when a few cubic centimeters of the solvent were evaporated to dryness. The extracts obtained have not been investigated as to their composition.

The percolates from the petroleum ether, ether, chloroform and alcohol extractions were placed in large evaporating dishes and the solvent allowed to evaporate spontaneously or by gentle warming, if necessary.

The filtrates from the hot water extractions were placed in evaporating dishes and heated on the steam bath until the water was removed. The filtrates from the acid extractions were neutralized with aqueous sodium hydroxide; the precipitates which formed upon neutralization (if any) were filtered off and dried; the resulting filtrates were then evaporated under reduced pressure. The filtrates from the alkali extractions were neutralized with hydrochloric acid; the precipitates which formed upon acidification (if any) were filtered off and dried; the filtrates obtained were then evaporated under reduced pressure.

(*a*) *The Marc.*—The residue remaining after this treatment was a tasteless, odorless, tan powder which still contained a considerable number of coarse particles. These particles were probably pieces of stem which were unavoidably included with the flower-leaf portion during the mechanical separation of the stems from the flowers and leaves.

The total extraction time and the percentage of extracted material obtained with each solvent are listed in Table V. The weights of the acid extracts and the alkali extracts were found by the differences in the weights of the air-dried samples before and after the respective extractions. This obviated the difficulty involved in determining accurately the amount of sodium chloride present as a contaminant in the acid and alkali extracts.

Table V.—Extraction with Selective Solvents

Solvent Employed	Number of Extractions	Extraction Time in Hours I	II	III	Per Cent of Extract[a] I	II	III
Petroleum ether	Continuous	82.75	83.00	82.75	4.51	4.21	4.22
Ether	Continuous	229.00	227.75	227.75	13.75	15.50	13.54
Chloroform	Continuous	101.50	101.50	101.50	1.94	2.24	2.04
Alcohol	Continuous	411.75	414.75	414.50	11.43	12.16	11.24
Water	31	108.50	108.50	108.50	24.80	24.73	24.75[b]
Acid	15	140.25	140.00	140.25	17.19	16.93	17.66
Alkali	11	254.25	254.25	254.25	15.51	15.99	14.65
Marc	..	....	....	....	6.61	6.21	5.53
Water in air-dried plant	..	....	....	....	7.36	7.34	(7.35)[c]
Totals[d]					103.10	105.31	100.98

[a] The percentages of extract are based on the weight of the air-dried, flower-leaf portion of the plant taken for the samples.
[b] A few grams of the sample were lost during this extraction as the result of an accident.
[c] This value was not determined; it represents the average value of Samples I and II.
[d] The excessive totals may be accounted for by hydrolysis or oxidation of components or the incomplete removal of solvents.

C. THE VOLATILE OILS

1. DISTILLATION OF THE FRESH PLANT

As was previously indicated, 85.61 Kg. (112.37 Kg. green weight) of the 1938 crop and the total 1939 crop of 186.10 Kg. were set aside for steam distillation. This was carried out by charging a large, steel steam-still with about 10 Kg. of the fresh plant and distilling during a period of from two to three hours. The steam used was taken directly from a line carrying steam under a pressure of thirty pounds per square inch.

The distillate was collected in a Florentine receiver. A total volume of 70 cc. of reddish brown oil separated from the distillate obtained from the 1938 plant. From the steam distillation of the 1939 crop, a total volume of 204 cc. of oil, darker in color than the 1938 oil, was obtained. Approximately

twenty liters of aqueous distillate were collected from each charge of fresh plant. The aqueous, oil-saturated distillates were definitely acidic to litmus. Cohobation of these distillates, under both reduced and atmospheric pressure, failed to yield any additional oil.

The oil which separated spontaneously from the aqueous distillates was removed from the Florentine receiver and dried over anhydrous sodium sulfate.

The aqueous distillates, after separation of the oil therefrom, were extracted with petroleum ether in order to obtain whatever oil had dissolved in the water; ninety liters of the distillate were not extracted but set aside for possible future investigation. The extraction was carried out by placing about ten liters of the distillate and two liters of petroleum ether in a twenty-liter bottle, stoppering securely, and agitating for one-half hour on a shaking machine. The aqueous layer was then removed and discarded. The petroleum ether portion was used to extract the next portion of distillate. At the end of the extraction of the 1939 distillates, the petroleum ether layer had the appearance of an emulsion. About three hundred Gm. of anhydrous sodium sulfate were added to the emulsified petroleum ether. Upon absorption of water, the sodium sulfate solidified into a cake. This cake was allowed to dry by evaporation of the petroleum ether, powdered and extracted by percolation with several portions of petroleum ether. The extraction yielded, upon spontaneous evaporation of the solvent, 48 cc. of reddish brown oil somewhat lighter in color than that separating directly from the water. No difficulty was encountered with the petroleum ether extraction of the 1938 distillates; upon evaporation of the petroleum ether, 26 cc. of a reddish orange oil were obtained. The oils obtained are hereafter designated as the 1938 oil, the 1938 extracted oil, the 1939 oil and the 1939 extracted oil.

The percentage yields of the oils separating spontaneously from the aqueous distillates were as follows: 1938 oil—0.08 per cent; 1939 oil—0.10 per cent. The percentage yields of the oils extracted from the aqueous distillates were as follows: 1938 extracted oil—0.03 per cent; 1939 extracted oil—0.03 per cent. These percentage yields were based on the weight of plant upon its receipt in Lincoln.

2. SOME PHYSICAL PROPERTIES OF THE OILS

(*a*) *Specific Gravity.*—The specific gravity of the various oils was determined according to the usual method by use of a pycnometer. The specific gravities for the several oils are shown in Table VI.

(*b*) *Specific Optical Rotation.*—Ethereal solutions of the oils, prepared by dissolving 2.0 cc. of oil in enough ether to make 20.0 cc. of solution, were examined in 100-mm. tubes for determining the specific optical rotations of the oils. However, a 200-mm. tube was used for the 1938 oil. The values are shown in Table VI.

(*c*) *Refractive Index.*—The refractive indices of the oils, as determined by the use of an Abbé refractometer, are shown in Table VI.

Table VI.—Physical Constants of the Oils

	Specific Gravity	Specific Rotation	Refractive Index
1938 Oil	$0.9268\ \frac{25°}{25°}$	$-29.56\ \frac{20°}{D}$	$1.4868\ \frac{24°}{D}$
1939 Oil	$0.9332\ \frac{25°}{25°}$	$-19.69\ \frac{20°}{D}$	$1.4952\ \frac{20°}{D}$
1938 Ext. oil	$0.9727\ \frac{25°}{25°}$	$+\ 9.46\ \frac{20°}{D}$	$1.4805\ \frac{20°}{D}$
1939 Ext. oil	$0.9679\ \frac{25°}{25°}$	$+\ 0.25\ \frac{20°}{D}$	$1.4851\ \frac{20°}{D}$

(*d*) *Congealing Point.*—A congealing point apparatus of appropriate design was employed in an attempt to determine the temperature at which the oils would solidify. Solid carbon dioxide was used as the cooling agent. The temperature of the oil was allowed to drop to −60° C. (limit of thermometer) in each instance. The oils became very viscous at this temperature. However, none of the oils completely solidified; the 1938 extracted oil still flowed at this point. No crystals of any kind could be induced to separate from any of the chilled oils.

(*e*) *Solubility in Certain Solvents.*—It was found that only the 1938 extracted oil was soluble in alcohol. However, all of the oils were soluble in absolute alcohol, ether, petroleum ether, acetone and dioxane; the 1938 oil and the 1939 extracted oil were difficultly soluble in petroleum ether.

With the exception of the 1938 extracted oil, all of the oils contained some material which was insoluble in alcohol. When these were added to alcohol, precipitation occurred, expecially with the 1938 oil. The white precipitate which formed was filtered off, dried and its melting point determined. It was impossible to obtain a sharp melting point on this material; the range was from 93° to 140° C. Recrystallization from a dioxane-ether mixture failed to change materially the melting-point range.

The alcohol-insoluble material dried quickly, powdered easily and had no tendency to become gummy or sticky even under rather heavy pressure. Further investigation of this material has not been carried out.

3. SOME CHEMICAL PROPERTIES OF THE OILS

(*a*) *Reaction toward Litmus.*—Upon treating litmus paper with each of the oils, it was found that each oil caused blue litmus to become red, thereby indicating the presence of some acidic constituent.

(*b*) *Behavior with Alkaline and Neutral Permanganate.*—When the oils were shaken with cold, neutral or alkaline potassium permanganate, immediate decolorization of the permanganate solution occurred and a copious precipitate of manganese dioxide was produced. Upon acidification of the filtrate from the reaction mixture, no insoluble acid was produced. This would indicate the probable absence of compounds yielding, upon oxidation,

benzoic acid or its derivatives. The rapid decolorization of the permanganate solution would indicate the presence of some substance or substances that were easily oxidized.

(*c*) *Behavior with Bromine in Chloroform.*—Large quantities of bromine in chloroform, when added to the oils, were immediately decolorized. This would indicate the probable presence of a considerable proportion of unsaturated compounds.

(*d*) *Behavior with Nitrogen Oxychloride.*—Due to the odor of the 1939 oil, it was thought that the oil possibly contained pinene. Consequently an attempt was made to prepare the nitrosochloride derivative of pinene according to the method described by Gildemeister and Hoffmann (5). No crystalline material could be induced to separate from the reaction mixture. This would make it appear that there was little, if any, pinene in the 1939 oil.

(*e*) *Behavior with Metallic Sodium.*—When the oils were placed over freshly cut sodium, a very few bubbles made their appearance on the surface of the metal. There was no tendency for the continued evolution of hydrogen. Hence, the absence of any appreciable amount of hydroxyl groups capable of reacting with sodium has been shown.

4. CHEMICAL DETERMINATIONS ON THE OILS

(*a*) *Qualitative Elementary Analysis.*—The several oils were examined for the presence of nitrogen, sulfur and halogens. The usual sodium fusion reaction was carried out and appropriate qualitative tests performed on the aqueous alkaline filtrate obtained after leaching the fusion mixture with boiling water. Nitrogen, sulfur and halogens were shown to be absent.

(*b*) *Aldehyde and Ketone Determinations.*—(1) Qualitative Determination: Since, with the exception of the 1938 extracted oil, the various oils were insoluble in alcohol, a few drops of each oil were dissolved in about one cubic centimeter of toluene and to this solution was added a small amount of Schiff's Reagent. The presence of aldehydes in all of the oils was demonstrated.

Attempts to prepare phenyl hydrazone derivatives from the oils met with failure. Efforts to form semicarbazone derivatives were likewise fruitless.

(2) Quantitative Determination: A modification of the bisulfite method for determining the quantity of aldehydes and ketones as suggested by Gildemeister and Hoffmann (6) was followed. Due to the limited supply of oils available, 2.00-cc. samples were used and Babcock bottles were employed rather than using 10.00-cc. samples of the oils in cassia flasks.

At the end of the reaction period, the Babcock bottles were placed in the centrifuge and whirled at about fifteen hundred r. p. m. for twenty-five minutes in order to break the emulsion which had formed. The percentages of aldehyde or ketone or both by volume are shown in Table VII.

Table VII.—Percentages by Volume of Aldehyde-Ketone in Oils

	I	II	III
1938 Oil	10.0	10.0	11.0
1939 Oil	15.0	15.0	void
1938 Ext. oil	16.0	18.0	void
1939 Ext. oil	13.0	13.0	15.0

(*c*) *Phenol Determinations.*—(1) Qualitative Determination: The presence of a phenolic substance or substances was shown by Flückiger's (7) test for phenolic bodies.

(2) Quantitative Determination: The oils remaining from the aldehyde determinations were used in carrying out the quantitative estimation of the phenolic substance or substances. The oil was extracted with a three per cent aqueous solution of potassium hydroxide. The Babcock bottles were again used as substitutes for the usual cassia flasks. The diminution in the volumes of the oils was found and the percentages by volume of phenolic substances were calculated with reference to the original volume of oil, *viz.*, 2.00 cc. These percentages are listed in Table VIII.

Table VIII.—Percentages by Volume of Phenolic Substances in Oils

	I	II	III
1938 Oil	7.0	4.4	9.5
1939 Oil	1.8	1.6	15.8*
1938 Ext. oil	19.0	17.4	15.9
1939 Ext. oil	7.6	6.8	6.2

* No explanation for such a variation.

(*d*) *Acid Values.*—The acid values for the oils were determined according to the usual method. These values are shown in Table IX.

Table IX.—Acid Values of the Oils

	I	II	III
1938 Oil	7.46	7.14	7.30
1939 Oil	4.09	3.94	3.78
1938 Ext. oil	7.86	8.01	7.86
1939 Ext. oil	5.77	6.08	6.23

(*e*) *Saponification Values.*—The method of "The Pharmacopœia of the United States of America" (8) was followed in determining the saponification values for the various oils. The mixtures remaining from the acid value determinations were utilized in determining the saponification values which are tabulated in Table X. The odor of the titrated saponification mixtures resembled that of eucalyptol.

Table X.—Saponification Values of the Oils

	I	II	III
1938 Oil	29.96	38.90	36.02
1939 Oil	0.00	3.56	13.58
1938 Ext. oil	57.92	54.08	56.56
1939 Ext. oil	39.44	40.28	40.82

(*f*) *Ester Values.*—The ester values of the oils were found by subtracting the average acid value of an oil from the average saponification value for that same oil. These values are shown in Table XI.

Table XI.—Ester Values of the Oils

	1938 Oil	1939 Oil	1938 Ext. Oil	1939 Ext. Oil
Saponification value (av.)	34.96	5.72	56.18	40.18
Acid value (av.)	7.29	3.93	7.91	6.02
Ester value (av.)	27.67	1.79	48.27	34.16

(*g*) *Acetyl Values.*—The acetyl values of the four oils were determined according to the method of Gildemeister and Hoffmann (9). The acetyl values shown in Table XII were found by subtracting the average ester values of the non-acetylated oil from the average ester values of the oils after their acetylation.

Table XII.—Acetyl Values of the Oils

	1938 Oil[a]	1939 Oil	1938 Ext. Oil[a]	1939 Ext. Oil
Ester value (av.) of acetylated oil	161.04	117.81	206.20	190.08
Ester value (av.) of non-acetylated oil	27.67	1.79	48.27	34.16
Acetyl value	133.37	116.02	157.93	155.92

[a] One sample only.

(*h*) *Methoxy Values.*—Since methyl and ethyl ethers occur occasionally in volatile oils, an effort was made to determine the percentage of methoxy radical present in the oils. Perkin's modification (10) of Zeisel's method for the quantitative estimation of the methoxy group was employed. When the oils were added to the hydriodic acid, there seemed to be a liberation of iodine inasmuch as the mixture turned quite brown. Upon heating, however, the hydriodic acid layer became clear. Perhaps the iodine, if any were liberated, added on to some unsaturated linkage which may have been present. The oily layer turned dark brown to almost black as the heating of the mixture was continued. The methoxy values, as determined for the oils, are listed in Table XIII.

Table XIII.—Methoxy Values of the Oils[a]

1938 Oil	0.29
1939 Oil	0.03
1938 Ext. oil	None
1939 Ext. oil	0.58

[a] One sample for each determination.

These results would indicate that there were little ethoxy or methoxy components in any of the oils and none in the 1938 extracted oil.

5. FRACTIONAL DISTILLATION OF THE 1939 OIL

Since so little oil was available, only the 1939 oil was fractionally distilled under reduced pressure. Seventy-five cubic centimeters of the 1939 oil were placed in a long-neck, round-bottom flask into which was inserted a Hempel tube and an aspirator tube. A Brühl receiver was used to collect the various fractions. An oil bath was employed for heating the distilling flask. A water pump was used to reduce the pressure within the system to about twenty-five mm. of mercury. Seven fractions of oil were collected. Information concerning these fractions is tabulated in Table XIV.

(*a*) *The Various Fractions of Oil.*—Fraction 1 was a rather limpid, colorless oil having an eucalyptol-like odor. Fraction 2 was of a light straw color, slightly camphoraceous in odor and more viscous than fraction 1. Fraction 3 had somewhat of a camphoraceous odor, was slightly more viscous than fraction 2 and was also of a light straw color. Fraction 4 was straw colored, more viscous than fraction 3, but less camphoraceous in odor. Fraction 5 was greenish yellow, less viscous than fraction 4 and had an odor resembling that of dog fennel, *Anthemis Cotula* L. A bright bluish green color marked fraction 6; the odor and viscosity of this fraction was similar to fraction 5. Fraction 7 apparently contained considerable material resulting from thermal decomposition of the original oil. This last fraction was brownish green in color and the odor resembled that of burning rubber.

The residue which remained in the distilling flask had the appearance of rosin. It was hard, yet brittle. This material was removed from the distilling flask by using small portions of ether; alcohol was added to this solution whereupon precipitation occurred. The precipitate, when filtered off and dried, was tan to brown in color.

(*b*) *Some Physical Properties of the Fractions.*—The specific gravity of each of the seven fractions was determined by the use of a pycnometer. The refractive index of each oil was found by employing an Abbé refractometer and white light. These values are shown in Table XIV.

Table XIV.—Volumes and Some Physical Properties of the Fractions of the 1939 Oil

Fraction	Temp. Range, ° C.	Volume, Cc.	Sp. Gr. $\frac{25°}{25°}$	Ref. Index 20° C.
1	Up to 63	8.5	0.9278	1.4773
2	63–83	7.3	0.9548	1.4878
3	83–120	3.5	0.9613	1.4941
4	120–135	5.5	0.9508	1.4971
5	135–155	15.0	0.9394	1.5014
6	155–165	7.0	0.9620	1.5016
7	165–168	4.5	0.9716	1.5019

(*c*) *Some Chemical Properties of Some of the Fractions.*—As was previously pointed out, the odor of fraction 1 was strongly suggestive of eucalyptol. Eucalyptol was tested for according to the methods of "The Pharmacopœia of the United States of America" (11) and of Baeyer and Villiger (12). The presence of eucalyptol could not be demonstrated in spite of the resemblance in odor. The specific gravity of fraction 1 was almost identical with that of eucalyptol; however, the refractive index disagreed with that for eucalyptol.

Fraction 6 possessed a bluish green color. The possibility of the presence of azulene, a blue hydrocarbon, was considered. Sherndal (13) considered the formation of a crystalline picrate as the best method of identifying azulene. Sherndal's method (14) was applied to fraction 6 with negative results.

A portion of the residue which remained in the distilling flask was tested for the presence of rosin using Liebermann's method (15). Rosin was found to be absent.

SUMMARY

A partial chemical investigation of *Artemisia filifolia* Torr. has been made. Data are reported for moisture, volatile material and ash. The results of extraction of the flowers and leaves with selective solvents according to a modification of Dragendorff's method are given.

Steam distillation of the fresh plants in 1938 and 1939 yielded 0.08 and 0.10 per cent, respectively, of volatile oils which separated spontaneously from the aqueous distillate. Oils extracted from the aqueous distillates of 1938 and 1939 with petroleum ether amounted to 0.03 per cent in each case. The total yield of oil was therefore 0.11 per cent for 1938 and 0.13 per cent for 1939.

Certain physical and chemical constants and properties of the volatile oils are reported. Slight variations in the physical constants of the oils are to be expected inasmuch as the 1938 crop was harvested in October while the 1939 crop was collected in early August. The specific gravity and the refractive index of the 1938 oil were less than those of the 1939 oil; on the other hand, the specific optical rotation was greater in the 1938 oil than in the 1939 oil. The constants and properties reported include specific gravities; specific optical rotations; refractive indices; congealing points; solubilities in certain solvents; behavior with potassium permanganate, bromine and nitrogen oxychloride; qualitative elementary analysis; quantitative determination of aldehydes-ketones and phenols; acid, saponification, ester, acetyl and methoxy values.

Fractional distillation of the 1939 volatile oil yielded seven fractions of oil which are described. The specific gravities and refractive indices of these fractions were determined. One fraction possessing the odor of and having a specific gravity almost identical with that of eucalyptol failed to give positive evidence of the presence of eucalyptol. Another fraction was blue-green in color, suggesting the presence of azulene which was shown to be absent.

Since this is only a preliminary chemical study of *Artemisia filifolia* Torr., additional investigation will be necessary before a complete phytochemical report on *Artemisia filifolia* Torr. can be made.

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"Those who never retract their opinions love themselves more than they love the truth"—Joubert

Pharmaceutical Emulsions. I A Study of the Continental Method*,†

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INTRODUCTION

The theory and practice of emulsification have been widely studied in various fields. However, the time-honored emulsification techniques of pharmacy have perhaps not received as much scientific study as might have been desirable.

The present investigation was undertaken with a view of making a detailed study of the importance of various factors in the technique used in the English and Continental methods, employing various oils. These older methods were also compared in effiency with newer methods, *i. e.*, the use of the electric mixer and hand homogenizer. The quality of the emulsions was determined by making measurements of the size of the dispersed globules, using a filar micrometer, as well as by observing the appearance and rate of creaming. Likewise, in some instances, photomicrographs were taken to show the quality of the emulsion.

The present paper gives the results of a study of the Continental method of emulsification.

EXPERIMENTAL

Materials Used.—The fixed oils selected for study were cod liver oil, castor oil, heavy mineral oil and linseed oil; the oils were of U. S. P. quality. U. S. P. powdered acacia was employed. One-tenth per cent sodium benzoate was added to the distilled water to prevent mold growth in the emulsions while standing for observation.

General Methods.—The quality of the emulsions was determined by making microscopic measurements of the size of the dispersed globules, as well as by taking photomicrographs in some instances and by observing the appearance of the emulsions and the rate of creaming. In determining the average diameter of the oil globules a filar micrometer was employed. A slide was made from each emulsion by placing a drop of the emulsion on a slide and then placing a cover glass over it. In some cases it was necessary to dilute the emulsion slightly with water in order to obtain a field suitable for observation. A representative field was chosen for observation, using the 4-mm. objective, and the diameters of all the oil globules touching the lines on the scale were recorded. In this manner, the diameters of about 50 oil globules were taken and the average globule size was determined. The average size of the oil globules was determined immediately after the completion of each emulsion.

Throughout the work, the rate of trituration was maintained at approximately 150 revolutions per minute. In all cases the parts of oil and water were measured in cc., and the parts of acacia were measured in Gm.

For observation the emulsions were kept in ordinary 2-oz. wide-mouth bottles at room temperature.

By the term "primary emulsion" is meant the emulsion which resulted when the oil and the acacia were triturated together and a portion of the water was added all at once. The primary emulsion was considered successful if it gave a clicking sound when triturated, was creamy, opaque and showed no oil separation. In some cases, especially where more than 2 parts of acacia or more than 3 parts of water per 4 parts of oil were used no primary emulsion was formed, but on gradual addition of the remainder of the water a homogeneous preparation resulted having the appearance of a temporary emulsion. The average size of the oil globules was determined in such cases.

In the tables "Oil Sep." is used to indicate oil separation. The following abbreviations are used to indicate the average size of the oil globules.

A—Average diameter less than 2.5 microns.
B—Average diameter from 2.5 to 4 microns.
C—Average diameter from 4 to 6 microns.
D—Average diameter more than 6 microns.

Variation in Proportion of Acacia.—In studying the effect of variation in proportion of acacia, the proportions of oil and water were kept constant. Sixty cc. of a 12½% oil emulsion was prepared in each case. The 4 parts of oil and the acacia were first triturated well for about a half minute, and then the 2 parts of water was added all at once. The primary emulsion was triturated for about 2 to 3 minutes and the remainder of the water was added gradually with constant trituration. A No. 1 wedgwood pestle, 17.5 cm. in length and 3.5 cm. in diameter at the base, and a No. 1 wedgwood mortar were used; in some instances a porcelain pestle, 15.0 cm. in length and 4.5 cm. in diameter at the base, was employed. The results of the effect of variation in proportion of acacia are given in Table I.

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* Presented before the Scientific Section, A. PH. A., Richmond meeting, 1940.

† This paper is based on part of a thesis presented to the Graduate Council of the University of Florida in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

‡ Head Professor of Pharmacy, University of Florida.

** Graduate Scholar, University of Florida, 1939–1940.

Table I.—Variation in Proportion of Acacia

Parts of Acacia[a]	Linseed Oil: Appearance of Emulsion, 1 Hour	Linseed Oil: Average Size of Oil Globules	Cod Liver Oil: Appearance of Emulsion, 1 Hour	Cod Liver Oil: Average Size of Oil Globules	Castor Oil: Appearance of Emulsion, 1 Hour	Castor Oil: Average Size of Oil Globules	Mineral Oil: Appearance of Emulsion, 1 Hour	Mineral Oil: Average Size of Oil Globules
(Wedgood Mortar and Wedgwood Pestle)								
4.0	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*C*
3.0	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*C*
2.4	Oil Sep.	*C*	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*
2.0	Creaming	*B*	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*
1.6	Creaming	*B*	Creaming	*B*	Oil Sep.	*D*	Oil Sep.	*D*
1.0	Creaming	*D*	Creaming	*B*	Stable	*C*	Oil Sep.	*D*
0.8	Oil Sep.	*D*	Creaming	*D*	Stable	*C*	Oil Sep.	*D*
0.6	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*
0.4	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*
(Wedgood Mortar and Porcelain Pestle)								
4.0	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*	Creaming	*B*
3.0	Oil Sep.	*C*	Oil Sep.	*D*	Stable	*A*	Creaming	*A*
2.4	Oil Sep.	*C*	Oil Sep.	*D*	Stable	*A*	Stable	*A*
2.0	Creaming	*B*	Oil Sep.	*C*	Stable	*A*	Stable	*A*
1.6	Creaming	*B*	Stable	*B*	Stable	*A*	Stable	*B*
1.0	Creaming	*C*	Stable	*B*	Stable	*B*	Stable	*C*
0.8	Oil Sep.	*D*	Stable	*D*	Stable	*C*	Creaming	*D*
0.6	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*
0.4	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*	Oil Sep.	*D*

[a] Number of parts of acacia used for 4 parts of oil and 2 parts of water.

Results of Table I show that emulsions prepared according to the 4:2:1 rule are not always best. When using smaller proportions of acacia a decrease in the amount of acacia caused an increase in the size of oil globules. Langevin (1), working with linseed, castor and cod liver oils, found similar results.

The use of a porcelain pestle of 4.5-cm. diameter showed a distinct advantage over a wedgwood pestle of 3.5-cm. diameter in that it caused a further reduction in the size of the oil globules and produced more stable emulsions. When using a wedgwood pestle, emulsions could not be formed when mineral oil was employed and with castor oil, emulsions were formed only when 0.8 and 1 part of acacia were used.

Variation in Time of Trituration of Primary Emulsion.—Making 60 cc. portions of 12½% oil emulsions, primary emulsions were triturated for various lengths of time using the 4:2:1 proportion and following the same procedure of preparation as in the previous experiment. A No. 1 wedgwood mortar and a No. 1 wedgwood pestle were employed in each case except the mineral oil emulsions which were prepared with the use of a porcelain pestle, 15.0 cm. in length and 4.5 cm. in diameter at the base, and a No. 1 wedgwood mortar. The results of variation in time of trituration of the primary emulsion are given in Table II.

Data of Table II show that when making 60 cc. of a 12½% cod liver oil emulsion, at least 4 minutes of trituration of the primary emulsion was necessary to produce an optimum finished product. Since further trituration of the primary emulsion seemed to be of no value, it was felt that 5 minutes of trituration should be ample to insure the best finished product. The results show that 5 minutes of trituration of the primary emulsion gave *A* products in the case of cod liver and castor oil with reference to the size of the globules. Linseed oil showed a grade *C* and mineral oil showed a grade *B* with 5 minutes of trituration.

Using the 4:2:1 proportion and a No. 3 wedgwood mortar and a No. 3 wedgwood pestle, eight ounces of cod liver oil emulsion was prepared to see if 5 minutes of trituration of a greater quantity of primary emulsion would also reduce the size of the oil globules in the finished product. It was found that the average size of the oil globules was grade *B* as compared to the 2 ounces of cod liver oil emulsion in which the average size of the globules was grade *A*. When making larger quantities of oil emulsions, it appears that more than 5 minutes of trituration of

Fig. 1.—Linseed Oil (430×).

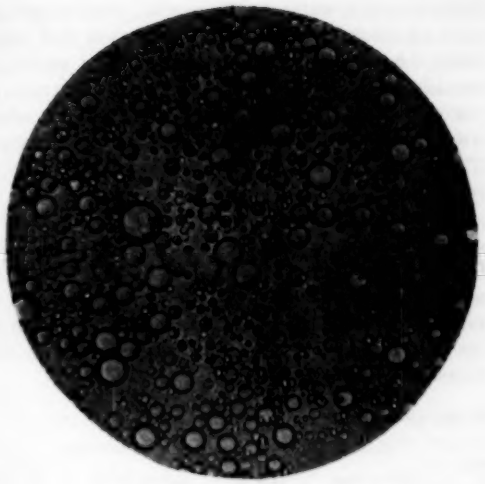

Fig. 2.—Mineral Oil (430×).

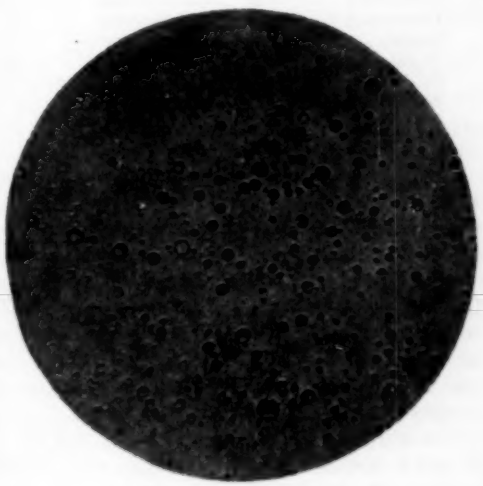

Fig. 3.—Cod Liver Oil (430×).

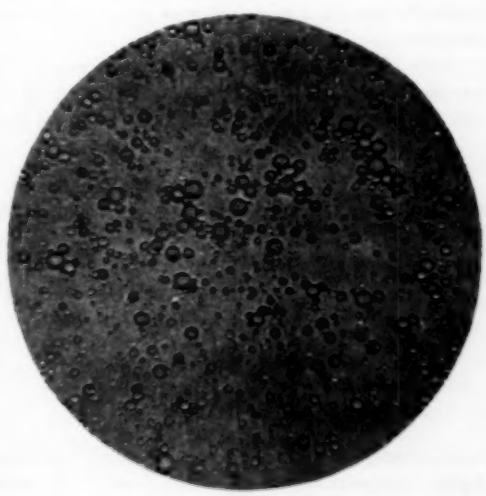

Fig. 4.—Castor Oil (430×).

the primary emulsion is necessary in order to produce an optimum dispersion.

Table II.—Variation in Time of Trituration of Primary Emulsion

	Time of Trituration in Minutes	Appearance of Emulsion, 3 Hours	Average Size of Oil Globules
Cod liver oil	1	Creaming	*C*
	3	Creaming	*B*
	4	Stable	*A*
	6	Stable	*A*
Castor oil	3	Stable	*B*
	5	Stable	*A*
Linseed oil	5	Creaming	*C*
	7	Creaming	*C*
Mineral oil	3	Stable	*C*
	5	Stable	*B*

Variation in Time of Trituration of Acacia and Oil in Making Primary Emulsion.—Most textbooks on pharmacy advise against excessive trituration of the acacia and oil before adding the 2 parts of water in the making of an emulsion by the dry gum method. It is generally thought that excessive trituration tends to make acacia colloidally soluble in the oil, thus favoring a water-in-oil rather than an oil-in-water emulsion. Sixty cc. portions of 12½% cod liver oil emulsions were prepared using the 4:2:1 proportion and using a No. 1 wedgwood mortar and a No. 1 wedgwood pestle. The acacia and oil were triturated for various lengths of time and the time of trituration of the primary emulsion was also varied in some instances. The remainder of the water was added gradually with constant trituration.

Table III.—Variation in Time of Trituration of Acacia and Oil in Making Primary Emulsion

(Cod Liver Oil)

Time of Trituration of Acacia and Oil	Time of Trituration of Primary Emulsion	Appearance of Emulsion, 3 Hours	Average Size of Oil Globules
1 minute	2 minutes	Creaming	*B*
3 minutes	2 minutes	Creaming	*B*
5 minutes	2 minutes	Creaming	*B*
5 minutes	5 minutes	Stable	*A*

The results show that as much as 5 minutes of trituration of the acacia with the oil did not seem to affect the finished emulsion; a grade *A* preparation, with respect to the size of the oil globules, was obtained when triturating the primary emulsion for 5 minutes.

Likewise, a muller was tried. One part of acacia was mixed well for about 2 minutes with a portion of the 4 parts of cod liver oil on a ground glass ointment slab using a muller. The pasty mass was then scraped off the ointment slab and placed in a No. 1 wedgwood mortar. The remainder of the 4 parts of cod liver oil was added and the entire mixture was triturated with a No. 1 wedgwood pestle for about another half minute. After the 2 parts of water was added all at once, the resulting primary emulsion was triturated for 5 minutes; the remainder of the water was added slowly with constant trituration.

The average diameter of the oil globules was about 2 microns. Better mixing of the oil and gum by the use of a muller was of no advantage.

Variation in Proportion of Water in Making Primary Emulsion.—In studying the effect of variation in proportion of water in making the primary emulsion, the acacia (1 part) and the oil (4 parts) were kept constant throughout the experiment. In all cases 60 cc. portions of 12½% oil emulsions were prepared using a porcelain pestle 15.0 cm. in length and 4.5 cm. in diameter at the base and a No. 1 wedgwood mortar. The procedure of preparation of the emulsions was the same as for Table I except that the primary emulsion was triturated for 5 minutes. Results are given in Table IV.

Table IV.—Variation in Proportion of Water in Making Primary Emulsion

	Linseed Oil		Cod Liver Oil		Castor Oil		Mineral Oil	
Parts of Water[a]	Appearance of Emulsion, 3 Hours	Average Size of Oil Globules	Appearance of Emulsion, 3 Hours	Average Size of Oil Globules	Appearance of Emulsion, 3 Hours	Average Size of Oil Globules	Appearance of Emulsion, 3 Hours	Average Size of Oil Globules
4.0	Creaming	*D*	Creaming	*D*	Creaming	*D*	Creaming	*D*
3.0	Creaming	*D*	Creaming	*B*	Creaming	*D*	Creaming	*D*
2.5	Creaming	*D*	Stable	*A*	Stable	*A*	Creaming	*C*
2.0	Creaming	*C*	Stable	*A*	Stable	*A*	Stable	*B*
1.5	None formed		None formed		Stable	*A*	None formed	
1.0	None formed		None formed		None formed		None formed	

[a] Number of parts of water used for 4 parts of oil and 1 part of acacia.

Only castor oil gave a primary emulsion when as little as 1½ parts of water was used; the other oils required at least 2 parts of water. When using more than 2 parts of water an increase in the amount of water caused an increase in the average size of the oil globules. When making emulsions using 4 parts of oil and 1 part of acacia, at least 2 parts of water and not more than 2½ parts of water should be used when making the primary emulsion to insure an optimum finished product.

Variation in Proportion of Acacia and Water.—Using the same size and type of mortar and pestle and the same method of preparation as in the preceding experiment, 60 cc. portions of 12½% oil emulsions were prepared. The proportion of oil (4 parts) was kept constant throughout. In all cases in the preparation of the primary emulsion twice as much water as acacia was used. Results of the effect of variation in proportion of acacia and water are given in Table V.

Table V.—Variation in Proportion of Acacia and Water

		Linseed Oil		Cod Liver Oil		Castor Oil		Mineral Oil	
Parts of Acacia[a]	Parts of Water[a]	Appearance of Emulsion, 3 Hours	Average Size of Oil Globules	Appearance of Emulsion, 3 Hours	Average Size of Oil Globules	Appearance of Emulsion, 3 Hours	Average Size of Oil Globules	Appearance of Emulsion, 3 Hours	Average Size of Oil Globules
1.0	2.0	Creaming	*C*	Stable	*A*	Stable	*A*	Stable	*B*
0.8	1.6	None formed		None formed		Stable	*A*	None formed	
0.6	1.2	None formed		None formed		Stable	*A*	None formed	
0.4	0.8	None formed		None formed		None formed		None formed	
0.2	0.4	None formed		None formed		None formed		None formed	

[a] Number of parts used for 4 parts of oil.

The results of Table V show that in the case of linseed, cod liver and mineral oils no emulsion was formed when using less than 1 part of acacia and twice as much water as the acacia; only castor oil gave an emulsion. In Table I it is seen that when using 0.8 part of acacia with 2 parts of water an emulsion was formed in several cases whereas in Table V only castor oil yielded an emulsion when using 0.8 part of acacia with 1.6 parts of water. The principle of using 2 parts of water for 4 parts of oil appears to be well founded.

Rate of Addition of Water in Making Primary Emulsion.—In making the primary emulsion, inferior results were obtained when the water was added slowly or in divided portions to the oil-acacia mixture. The principle of adding the water all at once in making the primary emulsion appears well founded.

Rate of Dilution of Primary Emulsion.—Experiments showed that after the primary emulsion had been prepared, the rate of addition of the remainder of the water had no effect on the finished product.

Direction of Trituration.—There has been some question as to whether the direction of trituration in the preparation of emulsions should be clockwise or counterclockwise. Experiments showed that the direction of trituration had no effect on the quality of the emulsion.

Use of Dried Acacia.—It was found that the use of dried acacia had no advantage over undried acacia in the preparation of emulsions.

Use of Wet Mortar.—The usual directions to use a dry mortar and pestle appear to be well founded, since the use of a moist mortar prevented emulsification.

Fifty Per Cent Emulsions.—Experiments were carried out with 50% oil emulsions to see whether or not the results obtained with the 12½% oil emulsions would be applicable to emulsions containing higher proportions of oil. The results of experiments with 50% oil emulsions were in agreement

with the findings on the 12½% oil emulsions, except that when making 60 cc. of a 50% cod liver oil emulsion at least 7 minutes of trituration of the primary emulsion was necessary in order to produce a grade *A* product. With mineral, castor and linseed oils, 8 minutes of trituration of the primary emulsion gave finished products which were similar, with respect to the size of the oil globules, to the 12½% oil emulsions in which the primary emulsions were triturated for 5 minutes. It was found that the 50% oil emulsions were more stable than the 12½% oil emulsions when standing over a period of three hours.

DISCUSSION OF RESULTS

Ingredients.—The present study of emulsions of various fixed oils, using acacia as the emulsifying agent, showed that the various oils behave differently with respect to range of emulsification, stability and the average size of the oil globules. Serrallach and Jones (2), studying the strength of interfacial films of various oils with emulsifying agents, pointed out that the influence of the oil on the properties of the film was more definite than that of the emulsifier. These workers showed that cod liver oil formed strong films very readily with most of the emulsifiers studied; castor oil had a lesser tendency to form films, and mineral oil had the least of any of the oils studied. In the present work, it was found that cod liver oil and castor oil gave grade *A* products with reference to the average size of the oil globules, using the 4:2:1 proportion, whereas mineral oil gave a grade *B* product and linseed oil gave a grade *C* preparation.

Time of trituration of the primary emulsion does not seem to be stressed in any of the pharmaceutical textbooks, and yet this factor is very important in production of the best emulsion. It was found that 5 minutes of trituration of the primary emulsion was ample to insure an optimum finished product when making 60 cc. of a 12½% emulsion. A greater quantity of emulsion requires longer trituration.

Roon and Oesper (3), using cottonseed and mineral oils in the Continental method found that the proportions giving the best results were 4 parts of oil, 2 parts of acacia and 3 parts of water and that slight variations from the proper procedure or from the critical proportions yielded either less stable emulsions or none at all. In the present work, when using mineral oil, a No. 1 wedgwood mortar and a No. 1 wedgwood pestle, no emulsions could be formed no matter what proportion of acacia was used. However, when using the wedgwood mortar and a porcelain pestle good emulsions were formed with several proportions of acacia. Castor oil gave grade *A* emulsions with various proportions of acacia when using a No. 1 wedgwood mortar and a porcelain pestle, but when using a No. 1 wedgwood mortar and a No. 1 wedgwood pestle emulsions were formed only when 0.8 and 1 part of acacia were used for 4 parts of oil and 2 parts of water. Stocking (4) found that castor oil produced poor emulsions.

The use of acacia dried to constant weight at 105° C. or placed over calcium chloride for 72 hours and the use of tears of acacia, powdered to a No. 80 fineness, did not seem to have any advantage over the commercial powdered acacia.

SUMMARY

A detailed study was made of the importance of various factors in the technique used in the Continental method of preparing emulsions.

Time of trituration of the primary emulsion was found to be a very important factor in production of the best emulsion. Castor oil and cod liver oil gave grade *A* products when using the 4:2:1 proportion, but it was necessary to use more than 1 part of acacia for emulsifying linseed oil and mineral oil in order to produce satisfactory preparations.

The principle of using 2.0 parts of water for 4 parts of oil and 1 part of acacia when making the primary emulsion is well founded. The use of a dry mortar and dry pestle is likewise important.

The following factors in preparation of emulsions did not have any effect on the finished product: the use of dried acacia, rate of dilution of the primary emulsion, excessive trituration of the oil and acacia when making the primary emulsion and direction of trituration of the primary emulsion, *i. e.*, clockwise, counterclockwise or both directions of trituration.

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(4) Stocking, C. H., JOUR. A. PH. A., 6 (1917), 954.

(*To be continued*)

Postage stamps have been issued by Italy in honor of the following: Leonardo da Vinci, painter, sculptor, architect and scientist; Alessandro Volta, electrophysicist; Luigi Galvani, physicist and anatomist.

Considerations for Evaluation of Germicides

*By Paul Goedrich**

In 1938 the author reported on new findings concerning the influence of p_H on the action of germicides (1).

On the four main types of antiseptics: the mercurials, the phenols, the halogens and the oxidizers, evidence was presented which shows that a decrease in p_H always results in an increase in germicidal effect, an increase which is independant of the germicidal action of the agent used to lower the p_H. The results obtained by other investigators have confirmed these findings.

As this fact is now very well established, it is apparent that the mere naming of the concentration and the chemical nature of the active principle in the solution of the germicide will not give sufficient information about its performance. So, for instance, from a 5% phenol solution an effect on spore-bearing organisms is not expected, but when its p_H is lowered by the addition of small amounts of HCl, it displays a much better sporicidal action than a 20% formaldehyde solution.

A 7% U. S. P. Tincture of Iodine will not kill the spores of *B. subtilis* in two hours, whereas an acidified solution containing only 5% of iodine showed a killing action within 10 minutes. It was found that the same principle also applies for antiseptics in ointment form. An ointment containing 10% of ammoniated mercury showed a clear zone of 12 mm. on an agar cup plate infected with *Staph. aureus*, whereas the same concention of this antiseptic in the same medium showed a clear zone of 16 mm. when the p_H was lowered by the addition of a small amount of acid.

But, not only the p_H of a germicide must be considered in the evaluation of its action; also its "affinity" to organic matter like blood, serum, tissue fluid, mucous, etc., plays an important role.

Nye (2) of the Mallory Institute of Pathology demonstrated the fact that the commonly used germicides, except iodine, fail to exert a germ-killing effect when tested at body temperature in the presence of 50% horse serum. The application of the results of his findings is significant for the use of antiseptics in deeper wounds or body cavities, where there is always present a large amount of organic matter, which will counteract the effect of the germicide and may render it ineffective. Often we hear, even from very experienced surgeons, that they do not believe in the use of antiseptics and do not use them in their work. In the light of Nye's findings one can understand their position in this regard.

As Nye's results demonstrated the superiority of iodine solutions above other germicides in the presence of heavy organic matter, the author was interested to investigate the cause for this action. It has been previously found by the author that acidified iodine solutions show a higher germicidal effect than ordinary solutions of the same iodine content. As the ordinary iodine solutions have a lower p_H than the other commonly used germicides, the chemical interaction between iodine, serum and acidifying agents was investigated.

It was found that the acidified solutions, when titrated after they had reacted with a definite quantity of serum, retained a much higher amount of available iodine than ordinary iodine solutions of the same iodine content. This reaction seems to be applicable also to solutions of the other halogens, because it was found that when Chlorine Water was acidified and tested in the presence of serum in the same way as the iodine solutions, a similar retention of chlorine occurs.

Various acidifying agents have been used for the reduction of p_H with the same effect. For instance: Diluted Lugol Solutions of about 2% titratable iodine content, acidified with various amounts of HCl, have been tested in comparison with regular Lugol Solutions of the same iodine content in the presence of a definite amount of serum. In the acidified solution free iodine is retained for from 5 minutes to three hours longer, according to the amount of acid used to lower the p_H. Lugol Solutions acidified

* From the Research Laboratories of the New Jersey College of Pharmacy, Rutgers University.

with hydriodic acid or oxalic acid showed a similar effect.

Freshly prepared chlorine solutions in water, acidified with a small amount of diluted sulfuric acid, extended the action of chlorine in the presence of an abundance of serum for a period ten times as long as that observed for unacidified solutions of the same chlorine content. A more detailed report on the effect of low p_H germicides, in the presence of heavy organic matter, will be given in the near future.

REFERENCES

(1) JOUR. A. PH. A., 27 (1938), 1233.
(2) *J. Am. Med. Assoc.*, 108 (1937), 280.

Book Reviews

Chemicals of Commerce, by FOSTER DEE SNELL and CORNELIA T. SNELL, viii + 532 pages, 5½ x 8½. 1939. New York: D. Van Nostrand Co. Price, $5.00.

In this volume, all chemicals offered on the market in sufficient quantity or usefulness to justify their consideration are discussed. There are short monographs on each chemical giving practical information on its general properties and uses. The monographs on related materials are grouped into chapters. It is believed that the book will serve the pharmacist as a useful source of information which is not available to him in pharmaceutical publications.—A. G. D.

The Story of Vitamin B_1, compiled by C. R. ADDINALL, Merck and Company, Rahway, New Jersey Revised Edition, 1940, 72 pp.

The revised edition of this booklet follows the same arrangement as the first edition (1937) and maintains the same high degree of excellence in telling the story of vitamin B_1. It is well documented with hundreds of references to the original literature, but it is more than a bibliography. It presents in a terse and interesting manner a résumé of the available information concerning the isolation and synthesis of vitamin B_1, its properties and methods of standardization. The chapters relating to the physiological action of thiamin and its role in diet and nutrition have been brought up to date by additional information and literature citations. The chapters on vitamin B_1 in plant growth should be of special interest to biochemists and plant physiologists. The booklet possesses a well-planned and entirely satisfactory index. It is not for sale, but is supplied only to those who have use for the information presented.—J. L. P.

History of Pharmacy—A Guide and Survey, by EDWARD KREMERS, Ph.G., Ph.M., Ph.D., Sc.D., Former Director, of the Course in Pharmacy and Professor of Pharmaceutical Chemistry, University of Wisconsin, author, editor and historian, and GEORGE URDANG, Ph.G., Sc.D., Nat. Honorary Member of the AMERICAN PHARMACEUTICAL ASSOCIATION, Former Editor of the *Pharmazeutische Zeitung*, Former Director of the Society for the History of Pharmacy, Berlin, author and historian. Thirty illustrations. Published by J. B. Lippincott Co., Philadelphia, London, Montreal. Price $4.50.

The senior author states in the preface that he has desired and had the intention for a number of years to write a history of pharmacy—for pharmacists. He states that well-meaning friends have prodded him on to the task, and he ascribes insufficient time and energy rather than lack of willingness to undertake the work. Evidently the opportunity was presented to him by his co-worker and colleague, Dr. George Urdang, to whom Dr. Kremers gives credit for preparing the plan and continuation of studies. Dr. George Urdang has made the acquaintance of many American pharmacists and has participated in the activities and proceedings of the profession in this country. Before leaving Berlin, he collaborated with Dr. A. Adlung in the publication of a volume of the History of Pharmacy and the German National Pharmaceutical Association. In this connection, the reviewer suggests to the readers the re-reading of the "Introductory Lecture to a Course in the History of Pharmacy" by Dr. Kremers and the discussion following on pages 1270–1279 of the JOURNAL of the A. PH. A. for 1933 and a study of the preface to the volume under review.

The contents are divided into four parts in the order of periods and developments to which notes are given in the following: Part I, Early Backgrounds in the Old World; Ancient Civilization and Ages. Part II, The Rise of Professional Pharmacy in Europe; Medical Theories and Materia Medica—the Developments in Italy, France, Germany, England; International Trends. Part III deals with the history of pharmacy in the North American Colonies up to the Revolutionary War and brings out the events of the Young Republic and Pioneer Expansion; Growth of Associations—Local, State, National; History of Legal Regulation; Development of Pharmaceutical Education; Establishment of a Pharmaceutical Literature; Relation of the Pharmacist to Society, etc. Part IV is devoted to a review of the contributions of pharmacists to science and industry. Each chapter contains much that is informative and interesting. In addition, volumes of information are presented bibliographically, which adds greatly to the usefulness of the book to students and teachers and as a historical record. American pharmacists and those of Europe know the senior author, and the co-worker is becoming well and favorably known to American pharmacists, as heretofore stated. It is, therefore, unnecessary to extend the length of this review. Suffice it

to say that the authors have contributed a valuable service by making available to students of pharmacy and others a wealth of historical information on the development of pharmacy in this country.—E. G. E.

Applied Pharmacology, by HUGH ALISTER MCGUIGAN. . 914 pages. St. Louis: The C. V. Mosby Company, 1940. Price, $9.00.

This interesting handbook on pharmacology contains more than nine hundred pages and many illustrations. To the reviewer the most striking feature of this work is the peculiar and almost extraordinary arrangement of subject matter, the book differing from most texts in not being divided into chapters but having instead a great many sections with headings in large and small bold-faced type. It would stretch one's candor too far to state that the classification employed is a logical one; in fact, in grouping or subdividing various subjects, the book follows no special criterion as do most texts on pharmacology. Some of the subdivisions have a solely physiological and others a chemical basis. Still others are purely pharmaceutical in character while there is a sprinkling of topics which cannot be classified at all. Thus, for instance, "crocodile tears" are relevant to no strictly pharmacological subject although the bit of information given under this title is of interest from the standpoint of popular science. Discussed under strictly physiological headings are the effects of drugs on respiration, on the heart and circulation, on the central nervous system, on the kidney, the liver, the uterus and the muscles; on salivary glands, on deglutition, absorption through the skin, vision and other physiological functions but intermingled with this rational physiological classification are topics of purely chemical nature such as the acids, alkalis, halogens, dyes and similar groups. Again, many of the sections bear such purely pharmaceutical titles as astringents, soaps, gargles, expectorants and hypnotics. On the whole, however, the book contrives to cover fairly well the whole field of pharmacology as it is taught in the average medical school, and it is up-to-date in that it includes adequate discussions on chemotherapy, vitamins, hormones and proteins (sera, etc.) in relation to therapeutics. Many well-known pharmaceutical preparations, such as mercurochrome, for example, receive no other notice than a listing in the long catalogs of materia medica contained in this volume. The book has many admirable features. Thus, for instance, there is no cluttering of the pages with abstruse chemical formulas. Some of the diagrams are very instructive and enlightening and the text is generally clear although the literature throughout is scanty and inadequate.—D. I. M.

NOTICE

A. PH. A. Research Fellowships

The Committee on Pharmaceutical Research of the AMERICAN PHARMACEUTICAL ASSOCIATION, announces the availability of certain limited funds for the establishment of research fellowships for the school year 1941–1942, and invites applications for these grants. The allowance in each case is usually from $300 to $400.

These grants are to be made by the Council of the AMERICAN PHARMACEUTICAL ASSOCIATION on the recommendations of the Committee on Pharmaceutical Research. They are to be made on the following basis:

(a) The extent to which the award will serve to promote Pharmaceutical Research.

(b) The extent, if any, to which the work supplements the program of the AMERICAN PHARMACEUTICAL ASSOCIATION'S Laboratory.

(c) The qualifications of those who will perform the work for which the award is made and the facilities of the laboratory wherein the research will be conducted.

(d) Preference will be given to applications wherein it is indicated that the award will supplement a contribution from the institution or laboratory in which the research will be conducted.

Application blanks may be obtained from the Chairman of the Committee on Pharmaceutical Research (Francis E. Bibbins, R.R. 16, Box 728-B, Indianapolis, Indiana). These blanks with all details necessary to evaluate the projects must be returned to the Committee not later than May 15, 1941.

Announcements of the awards will be made early in the summer.

Signed: Francis E. Bibbins, *Chm.*